VOLUME 81 *June 1960* NUMBER 6

THE AMERICAN REVIEW OF RESPIRATORY DISEASES

Clinical and Laboratory Studies of Tuberculosis and Respiratory Diseases

OFFICIAL JOURNAL OF AMERICAN TRUDEAU SOCIETY
MEDICAL SECTION OF THE NATIONAL TUBERCULOSIS ASSOCIATION

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(Formerly THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES)

Published Monthly at Mount Royal and Guilford Avenues, Baltimore 2, Maryland, by the National Tuberculosis Association. Business Off., 1790 Broadway, New York 19, N. Y.

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Medical Section of the National Tuberculosis Association

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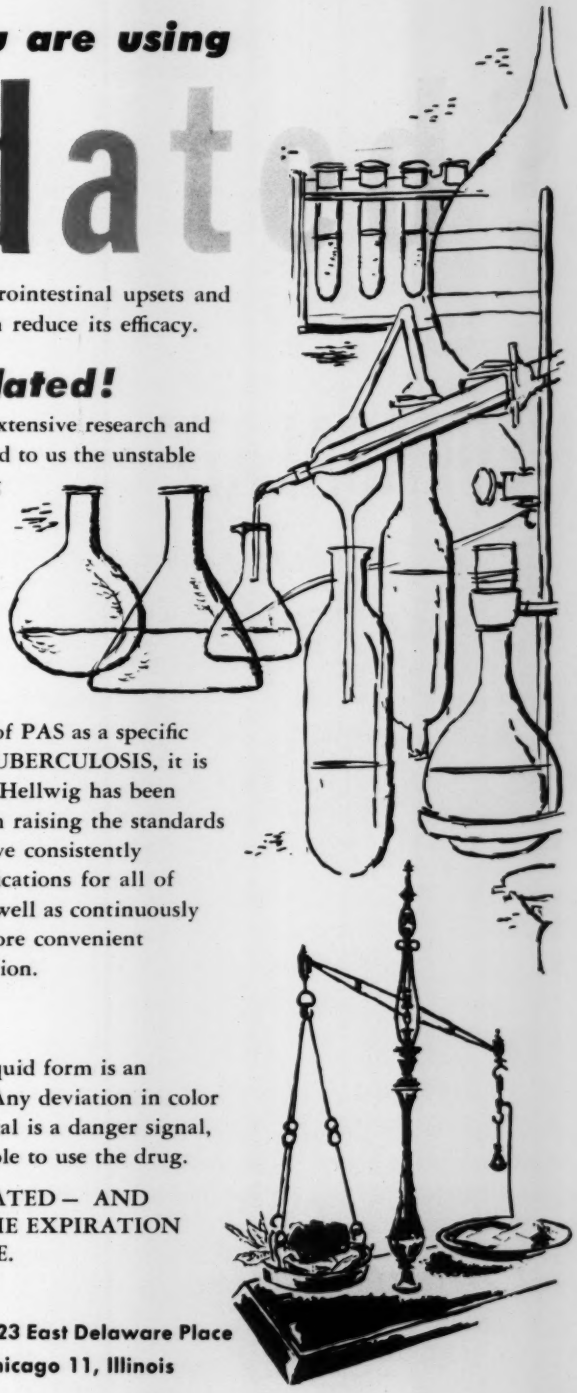

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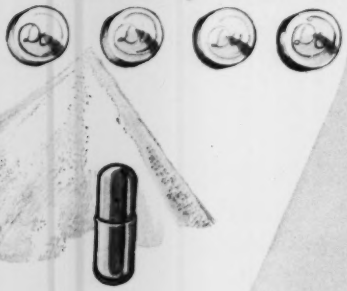

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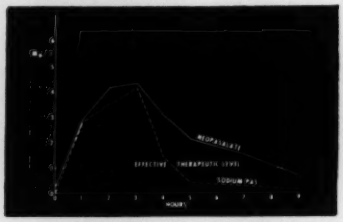

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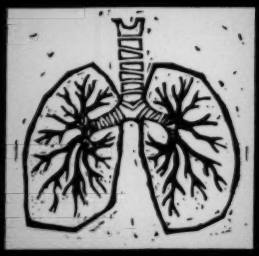

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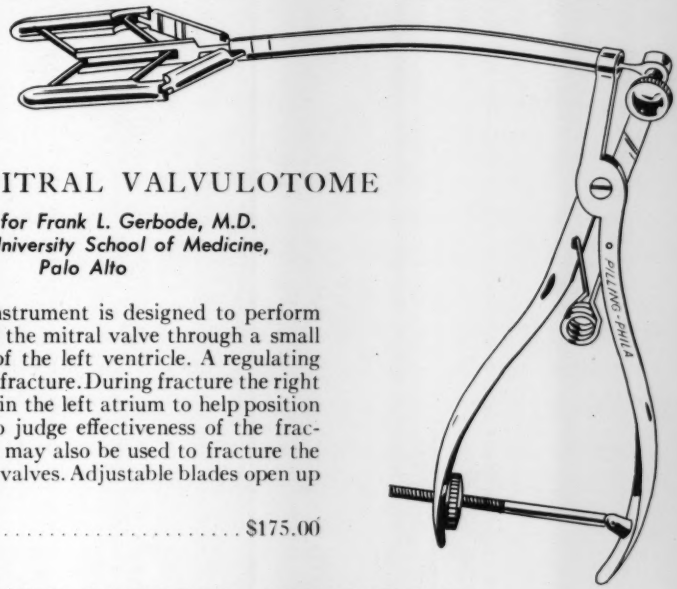

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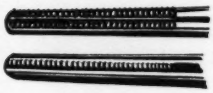

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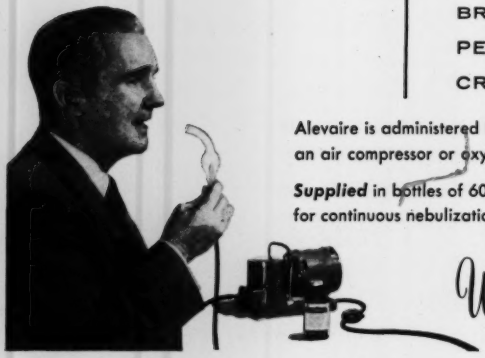

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VOLUME 81 *June 1960* NUMBER 6

THE MANAGEMENT OF THE TETRALOGY OF FALLOT WHEN COMPLICATED BY PULMONARY TUBERCULOSIS[1]

REEVE H. BETTS, T. THOMAS, AND N. GOPINATH

(Received for publication October 5, 1959)

INTRODUCTION

The occasional association of active pulmonary tuberculosis and congenital cyanotic heart disease is to be expected. As tuberculosis is comparatively common in the general population of India, and as there is ample evidence that congenital cardiac defects are as frequent here as elsewhere, the association of such lesions is thus to be anticipated. Although brief reference to the problem has been made by Basu (1) and the present writers (2) in the Indian literature, the only detailed studies found in the recent literature have been by the group at Johns Hopkins Hospital in the United States (3–6). These investigations indicate that it is not just the problem of a cardiac lesion complicated by pulmonary disease, but that the actual management of the cardiac defect may need to be modified.

Up to January 1, 1959, in the present study, 10 cases of associated active pulmonary tuberculosis and cyanotic heart disease have been encountered among a total of 173 patients diagnosed as having some form of the tetralogy of Fallot (figure 1). In 3 of the 10 cases, one of which is cited below, no surgical procedure was carried out. In the other cases, various methods of treatment were employed. Some of the deleterious effects previously reported to follow systemic pulmonary arterial anastomosis in the presence of pulmonary tuberculosis have not been encountered in this series.

CASE REPORTS

Case 1. Mr. C. (C.M.C.H., 67682), a 37-year-old male, who was admitted to the hospital on December 13, 1958, gave a history of dyspnea and a tendency to squat since early childhood. He stated that cyanosis had not been noticed before the age of twenty. In the past few years, all of his symptoms were found to be increasing at a rapid rate, and at the time of admission he was able to walk only a few yards. Cough, fever, and production of sputum were first noted two years before admission. At the time of admission his sputum was negative for tubercle bacilli. The hemoglobin was 22 gm. A roentgenogram of the chest (figure 2) revealed bilateral pulmonary tuberculosis. He was started on tuberculous chemotherapy and advised to report later for possible treatment of his congenital heart lesion.

Comment: The above case is typical of 3 of the 10 patients who were found to have associated active tuberculosis and congenital cyanotic heart disease and who were given chemotherapy and advised to return later for possible surgical consideration. It has not been possible to carry the plan out, as the tuberculosis has never been brought to a stable state in one patient, and the other 2 patients have not returned.

Case 2. Mr. M. (C.M.C.H., 60823), a 17-year-old male, was admitted to the hospital on June 13, 1958, with a history of cyanosis from birth and increasing dyspnea on exertion. Examination revealed quite typical findings of tetralogy of Fallot with a right aortic arch. Examination of the blood showed 20 gm. of hemoglobin. A left thoracotomy was carried out on July 14, 1958, but, on opening the left thorax, it was found that the pleura was studded with small tubercles, some of which were removed for pathologic study. Because of the marked involvement of the pleura along the mediastinum, it was considered unwise to continue with a shunt type of operation in the presence of this untreated tuberculous lesion. The operation was, therefore, terminated. The patient had a rather "stormy" postoperative course for the first week, but was discharged in good condition on chemotherapy on August 9, 1958. He

[1] From the Cardio-Thoracic Unit, Christian Medical College and Hospital, Vellore, South India.

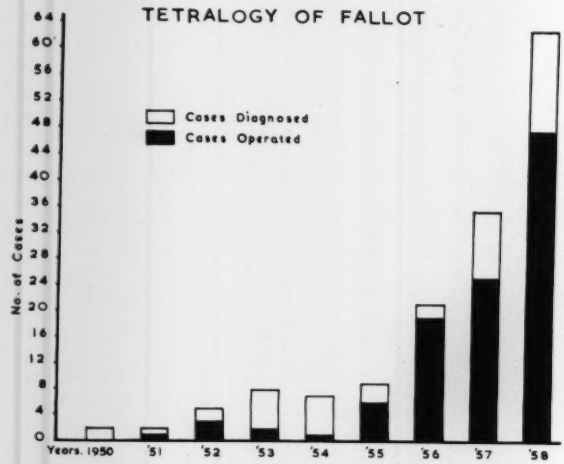

FIG. 1. Showing number of patients diagnosed and operated on for tetralogy of Fallot, 1950–1958. During this period there were 10 cases of associated active pulmonary or pleural tuberculosis.

was advised to come back for surgical consideration at a later date.

Comment: This is an example of a patient with unrecognized pleural tuberculosis until the time of operation, which had to be terminated because of this unexpected finding. A review of the preoperative roentgenogram (figure 3) showed no evidence whatsoever of any pulmonary lesion.

Case 3. Miss A. (C.M.C.H., 43463), an 18-year-old female, was admitted to the hospital on March 12, 1957. She complained of cyanosis with frequent fainting attacks since childhood. She found it necessary to squat frequently after moderate exertion. Clinical and laboratory investigations were compatible with the diagnosis of tetralogy of Fallot. The preoperative roentgenogram of the chest (figure 4) showed one or two suspicious shadows in the left lung field, but this finding was not seriously interpreted as clinical tuberculosis. On March 26, 1957, a left thoracotomy was carried out and it was obvious that there were tuberculous infiltrates in the upper lobe. The extent of the disease was only moderate and, as the pleura was not involved, it was decided to go ahead with the originally planned Blalock operation and trust to chemotherapy in the postoperative period to control the tuberculous lesion. The plan was adhered to, and the patient had an uneventful convalescence and was discharged on April 25, 1957, on tuberculosis chemotherapy. Her course since then has been very satisfactory and she has obtained a good result.

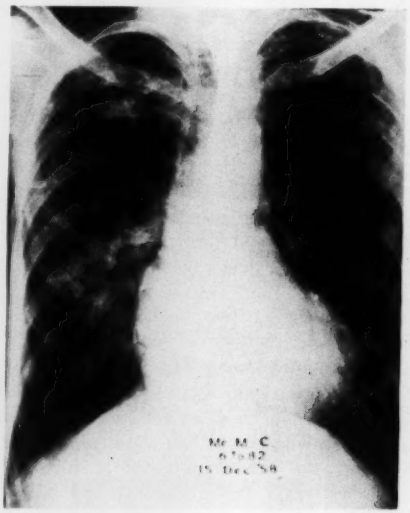

FIG. 2. *Case 1.* Mr. C. (67682). Roentgenogram showing the rather typically shaped heart in the tetralogy of Fallot. Moderately extensive bilateral tuberculosis is present which had not been previously diagnosed.

Comment: This patient, like the previous one, represents a missed diagnosis of tuberculosis until operation. It is apparently possible now, with the advantages of chemotherapy, to go

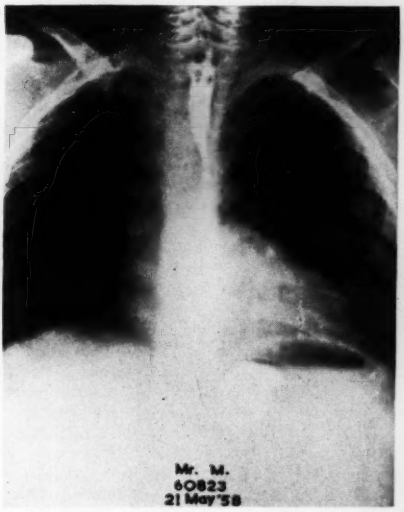

FIG. 3. *Case 2.* Mr. M. (60823). Roentgenogram showing no demonstrable pulmonary lesion. At thoracotomy the entire pleura was studded with tubercles.

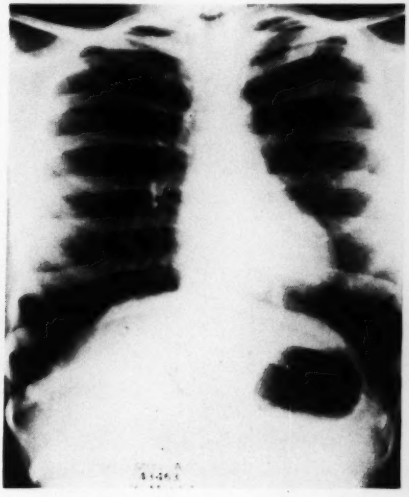

FIG. 4. *Case 3.* Miss A. (43463). Preoperative roentgenogram which on close scrutiny reveals two small areas of infiltration in left upper lobe that at operation were found to be tuberculous. A Blalock type of shunt was carried out, and with chemotherapeutic treatment the patient did well postoperatively.

ahead in the face of a moderate tuberculous lesion and rely on chemotherapy for control during the postoperative period.

Case 4. Miss J. (C.M.C.H., 70325), a 22-year-old female, was admitted to the hospital on February 26, 1959, and is stated to have had breathlessness and weakness since childhood and attacks of unconsciousness since November, 1958. Cyanosis had been present since birth and she was able to walk only an eighth of a mile on the level. Pulmonary tuberculosis was first diagnosed in October, 1954, and treatment with chemotherapy was given until March, 1955. The tuberculous process was limited to the right upper lobe on roentgenographic examination (figure 5). A diagnosis of tetralogy of Fallot with a left aortic arch was made. Cardiac catheterization revealed the pressure in the right ventricle to be equal to that of the systemic circulation. The hemoglobin was 15.5 gm. A left thoracotomy was done on March 14, 1959, when a large left pulmonary artery was found with a thrill palpable. In freeing up the various branches of the aortic arch and trying to trace back the origin of the thrill, it was finally discovered that there was a large patent ductus originating proximal to the origin of the left common carotid artery. The pericardium was opened, and the dissection continued until it was determined that this patent ductus apparently entered the left pulmonary artery just beyond its origin. In addition, just distal to the entrance to the ductus into the left pulmonary artery, there was a constriction of the pulmonary artery. The enlargement of the artery as seen at thoracotomy outside the pericardium was chiefly due to the post-stenotic dilatation effect that is encountered in such instances (figure 5*B*). No further procedure was carried out. The patient had a smooth postoperative course and was discharged on April 10, 1959.

Comment: The findings in this patient are illustrative of an abnormality not suspected before operation. A soft diastolic murmur had been heard along the left sternal border before operation, along with the louder systolic murmur, but no one had considered it at all suggestive of a patent ductus arteriosus. As nearly as could be determined at operation, the patent ductus supplied the left pulmonary artery primarily, and this was the side that was not involved by pulmonary tuberculosis. It might have been suspected from the meager literature (3–5) available that the high oxygen tension in the left pulmonary artery should have predisposed the tuberculous process to become established in the left lung rather than in the right.

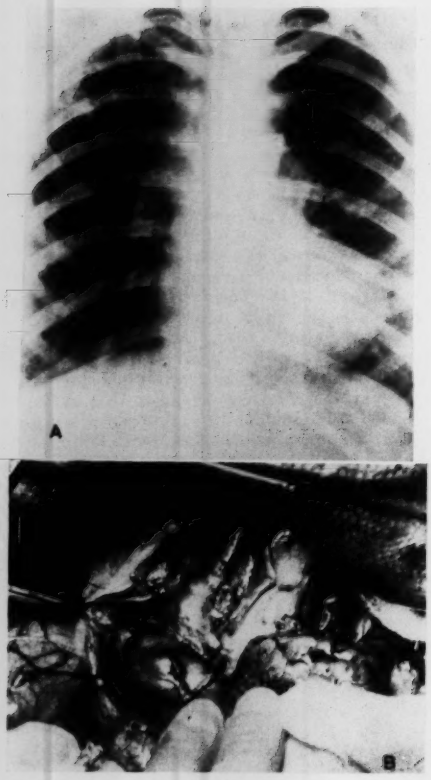

FIG. 5. *Case 4.* Miss J. (70325). (*A*) Preoperative roentgenogram showing tuberculous infiltration in right upper lobe after chemotherapy. There is a large left hilar shadow which was thought to be due to enlarged hilar nodes. (*B*) Photograph at operation demonstrating an enormously dilated left pulmonary artery (which caused the left hilar shadow) with a marked post-stenotic dilatation. The patent ductus originated proximal to the left common carotid and left subclavian arteries. (Subclavian shown in photograph.)

Case 5. Mr. J. (C.M.C.H., 56906), an 18-year-old male, was known to have had congenital heart disease since the age of five. A year and a half before admission to the hospital on March 1, 1958, he had a large hemoptysis and was discovered to have pulmonary tuberculosis. The sputum was positive for tubercle bacilli. Chemotherapy was given, but cavities persisted in both upper lobes, as shown roentgenographically (figure 6). No cyanosis was noted and the patient had a very loud systolic murmur. Cardiac catheterization revealed a pressure of 175 systolic, 5 diastolic in mm. of mercury in the right ventricle, with a pressure of 20 systolic, 5 diastolic in the main pulmonary artery. It was thought that this patient had pure pulmonic stenosis and pulmonary tuberculosis. A pulmonary valvulotomy was decided upon, and the patient was operated on on April 18, 1958. There was considerable difficulty in separating the lung on the left side, but bleeding was not excessive.

On carrying out the transventricular pulmonary valvulotomy, it was recognized that some other abnormality was present as well, as on one attempt the closed valvulotome did not enter the pulmonary artery and it was suspected that there was a ventricular septal defect with a dextraposed aorta. The pulmonary valve was divided and the patient stood the operative procedure quite well. His postoperative course also seemed to be satisfactory in the early period, but he died quite suddenly approximately twenty-four hours postoperatively. Post-mortem examination revealed that this was not pure pulmonic stenosis, but was actually a tetralogy of Fallot with a patent ductus arteriosus. The cause of sudden death was probably due to an injury of the aortic valve. Although the valvulotome had not been opened when the introduction was made that did not go into the pulmonary artery, apparently the closed blade cut one cusp of the aortic valve in going through. This produced a rather marked degree of aortic

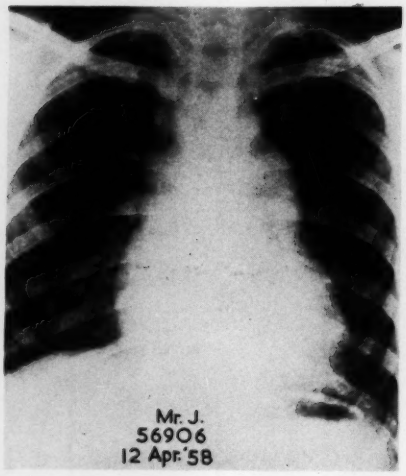

FIG. 6. *Case 5.* Mr. J. (560906). Roentgenogram demonstrating bilateral tuberculous infiltrates. The cardiac shadow was thought to represent "pure" pulmonary stenosis. A pulmonary valvulotomy was done, but the patient died within twenty-four hours. Post-mortem examination revealed a tetralogy of Fallot. A laceration of one of the aortic cusps had resulted in aortic regurgitation. A patent ductus arteriosus was also present.

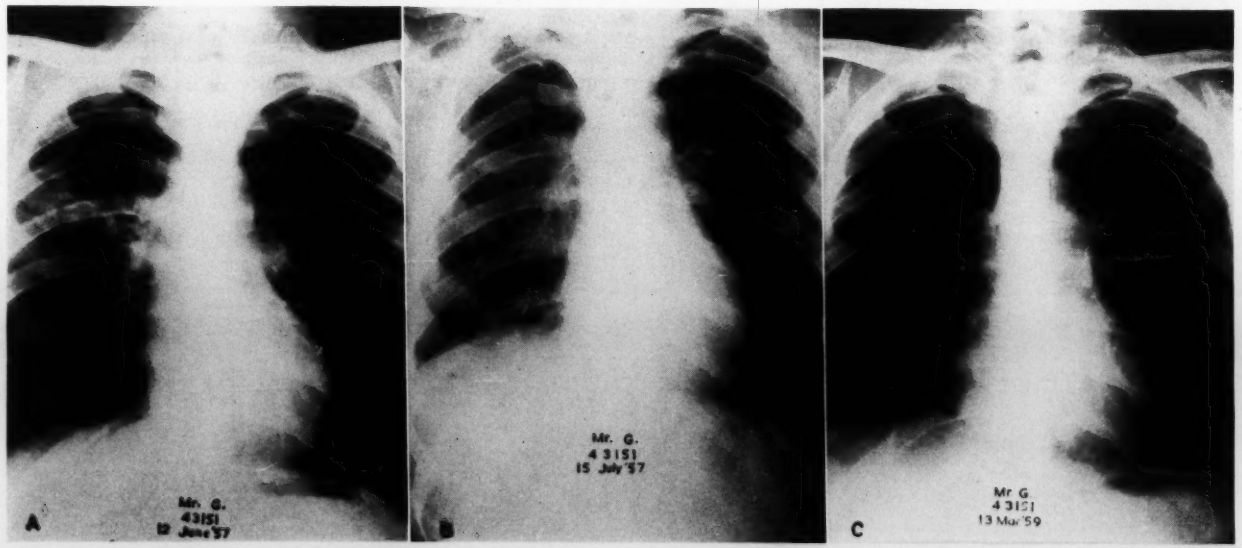

FIG. 7. (*Case 6*). Mr. G. (43151). (*A*) Roentgenogram, June 12, 1957, showing tuberculosis with cavity formation in right upper lobe. The cardiac silhouette is consistent with tetralogy of Fallot. (*B*) Roentgenogram, July 15, 1957, following right upper lobectomy. (*C*) Roentgenogram, March 13, 1958, after Blalock type of shunt operation on left.

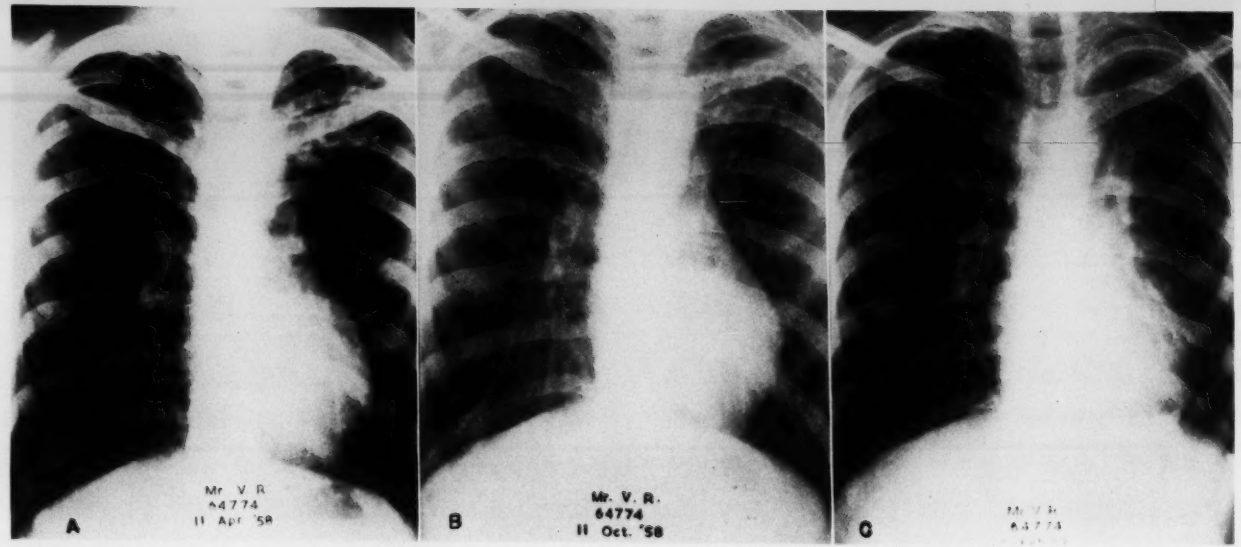

FIG. 8. (*Case 7*). Mr. V. (64774). (*A*) Roentgenogram, April 11, 1958, demonstrating tuberculosis with cavities in left upper lobe and a cardiac silhouette consistent with tetralogy of Fallot. (*B*) Roentgenogram, October 11, 1958, showing regression of tuberculous process under chemotherapy. (*C*) Roentgenogram, February 2, 1959, following left upper lobectomy and subclavian-pulmonary artery anastomosis at one time.

incompetence which, added to the other circulatory load he already had, was too much for him to survive. A marked amount of tuberculosis was found in both upper lobes with bilateral cavities. The post-mortem findings of patent ductus arteriosus explained the absence of cyanosis, but why no diastolic murmur was heard in view of the rather large patent ductus arteriosus is difficult to understand.

Comment: The presence of an associated patent ductus arteriosus should have been discovered on analysis of the samples at cardiac catheterization, but it was not. Pulmonary tuberculosis did not contribute directly to the fatal outcome in this case, unless perhaps by helping mask the diastolic murmur that should have been elicited preoperatively. Although a classic tetralogy of Fallot is easily recognized, it is not always true of some of the acyanotic forms.

Case 6. Mr. G. (C.M.C.H., 43151), a 25-year-old male, was first admitted to this hospital on March 2, 1957, at which time he had a history of being cyanotic since birth, but claimed he did not have any real dyspnea until the age of twelve. The dyspnea had been increasing and, in January, 1957, he had had his first hemoptysis. Pulmonary tuberculosis was not diagnosed, however, prior to admission. A chest roentgenogram revealed the lesion in the right upper lobe (figure 7). He was given tuberculous chemotherapy. It was decided that it would be best to try to control the pulmonary lesion first and defer decision regarding a shunt type of operation. On July 3, 1957, a right upper lobectomy was done (N. G.). The patient had a somewhat stormy postoperative course, with a temporary left hemiparesis which cleared spontaneously. He was discharged on July 26, 1958. On readmission on October 1, 1958, the tuberculous lesion appeared to be well controlled. There was no sign of any active lesion. On October 31, 1958, a Blalock operation was done on the left side (T. T.). His postoperative convalescence was essentially uneventful and he was discharged on November 21, 1958, and has since continued to be markedly improved.

Comment: This patient received what is probably the advisable form of treatment for a lesion of this type; that is, after chemotherapy the tuberculous lesion was controlled by surgery and then a shunt type of operation was done on the contralateral side.

Case 7. Mr. V. (C.M.C.H., 64774), a 20-year-old male, was admitted to the hospital on September 26, 1958, with a complaint of cyanosis since birth and increasing dyspnea on exertion. He had been a seven-month premature baby at the time of delivery. Pulmonary tuberculosis had been diagnosed in 1956, and he had had a large amount of chemotherapy. The lesion had not been controlled, however, and cavities were still present in the left upper lobe (figure 8). The patient was very cyanotic and had 22 gm. of hemoglobin. After much discussion it was decided to go ahead with a left upper lobectomy and simultaneous Blalock operation. This procedure was carried out on October 20, 1958, under moderate hypothermia. Vascular adhesions were found, but the upper lobectomy was carried out without great difficulty. As the patient's condition continued to be satisfactory, the Blalock shunt was completed. His postoperative course was a bit "stormy" for a day or two, but he soon settled down and was discharged on November 7, 1958. He was very markedly improved and has continued to do well since that time.

Comment: The advantage of removing the tuberculous process and completing the shunt operation at one sitting is attractive. No doubt there is some added risk in increasing the magnitude of the operation, but it also decreases the likelihood of getting trouble from the heart lesion if only the pulmonary lesion is taken care of at the first operation. Likewise, it eliminates the possibility of the worsening of the tuberculous process if the shunt operation only is done at the first sitting. It is perhaps justifiable in certain carefully selected cases to combine the two operative procedures.

Discussion

It has been stated (7) that the association of pulmonary tuberculosis and pulmonary valvular stenosis is rather common. Essentially the same conditions prevail in the lung in the case of "pure" pulmonary stenosis and in the tetralogy of Fallot, so far as blood flow and oxygenation are concerned. If tuberculosis and "pure" pulmonary stenosis are frequently associated, the same might be expected to apply to tuberculosis and the tetralogy of Fallot. The present writers thought that they had a case of tuberculosis and "pure" pulmonary stenosis as cited above (*Case 5*), but it proved to be a case of tetralogy of Fallot instead. Bjork and Bagger (7) and also Soutter and associates (8) have successfully carried out pulmonary valvulotomy and pulmonary resection simultaneously.

The human tubercle bacillus is an aerobe and grows best in an environment rich in oxygen. Much work has been done in attempting to correlate the site of predilection of tuberculosis in

animals and human beings on the basis of the oxygen tension in the blood in various parts of the lung. Medlar (9) conducted many experiments on rabbits along this line and was able to predict with considerable accuracy the probable site of infection.

It is to be expected, therefore, that, if a systemic artery were to be anastomosed to a pulmonary artery in a patient with pulmonary tuberculosis in the ipsilateral lung, the increased oxygen tension might have a deleterious effect on the tuberculous process. This is what was found in monkeys by Hanlon (6). The surprising thing is that the same group (8) found that ligating the pulmonary artery to a lung previously infected with tuberculosis also produced a rapidly progressing, caseous type of tuberculosis. Thus, in monkeys, whether the amount of oxygenated blood was decreased or increased, the tuberculosis was made worse. In the control animals, on which only a thoracotomy was done, no such changes were apparent.

On the basis of the above reports, Sloan and his associates (3) investigated the problem of tuberculosis and congenital cyanotic heart disease in a group of patients at the Johns Hopkins Hospital. They studied the first 1,000 patients operated on by Blalock and his associates for congenital cyanotic heart disease and found there were 39 who had tuberculosis on the basis of clinical examination, roentgenograms of the chest, and tuberculin testing. In this group there were only 17, however, who had disease of the reinfection type. It seems apparent from their report that in only two of these was active tuberculosis present at the time of the shunt operation. One additional patient died of tuberculosis before any cardiovascular operation was carried out.

Because of the experimental work cited above, Sloan and associates tried to avoid arterialization of the tuberculous lesion in the two patients with active pulmonary tuberculosis on whom they operated. In one patient they divided the left pulmonary artery and anastomosed the subclavian artery to the distal end, as the site of the tuberculous process was in the right upper lobe. The patient survived this operative procedure and was improved. But three years later, he still had sputum positive for tubercle bacilli so a lobectomy was carried out. Extremely vascular adhesions were encountered between the tuberculous right upper lobe and the chest wall; and, despite massive blood transfusions, the bleeding was not adequately controlled and the patient died ten hours after the operation. Their second case was a 21-year-old man with tuberculosis of six years' duration who had had a thoracoplasty performed on the right side, but continued to have an open cavity and sputum positive for tubercle bacilli. It was decided to do a right pneumonectomy with an end-to-end type of systemic pulmonary arterial anastomosis. Again, great difficulty was encountered with very vascular adhesions, and the patient died four hours after the operation was completed.

Mahoney (10) reported a 13-year-old patient with a tetralogy of Fallot who developed progressive tuberculosis after a Blalock operation on the left side. Later, a right upper lobectomy was carried out with success. Basu and Das (1) had a 28-year-old man with tetralogy of Fallot and tuberculosis of the right upper lobe. After six months of chemotherapy, a Blalock operation was done on the left.

It seems that individualization of treatment is necessary in patients with a combination of congenital cyanotic heart disease and active pulmonary tuberculosis. In the 10 patients of the present series, except for the 3 for whom chemotherapy was prescribed and who were expected to return and have not been operated on, the other 7, who have been operated upon, have all been treated differently. There are, however, several aspects that appear fairly clearly. First, if a patient is discovered with both diseases, the preferable method is to treat the tuberculosis by chemotherapy and, after it has become stable, to go ahead with surgical treatment of the heart lesion. Although each surgeon seems to have a special reason for using his own approach, it is generally true that a shunt type of operation can be carried out on either the right or the left side. It would, therefore, seem advisable, in view of the vascular adhesions that frequently are found in people with these two diseases, that the shunt operation be done on the side opposite the one that has the more extensive tuberculosis.

Second, for those patients who are discovered to have tuberculosis only at the time of thoracotomy, the local situation will have to determine the type of management. It would seem that to go ahead with an operative procedure, such as a vessel anastomosis, in the presence of active pleural tuberculosis would be hazardous and,

when this problem was encountered on one occasion, the operation was abandoned (*Case 2*). When the lesion is intrapulmonary and not very extensive, as it is likely to be when not picked up preoperatively, apparently the operation can be done with considerable safety under the protection of postoperative chemotherapy (*Case 3*).

Third, the present writers have not been strong advocates of pulmonary valvulotomy for the tetralogy of Fallot, even when a cone can be palpated, because it is associated so often with an infundibular stenosis as well. Nevertheless, there may be an occasional case in which a valvulotomy can be done with profit because of the shorter operating time and the avoidance of freeing up a larger area of very vascular adhesions. Fourth, it is difficult to know whether it is better to go ahead and control the tuberculous lesion or to alleviate the cardiovascular anomalies first in instances in which the tuberculosis cannot be arrested by chemotherapy. The writers have thought it best to control the infectious process first, but believe one should keep an open mind in regard to priority of treatment when confronted with a patient with active pulmonary tuberculosis and congenital cyanotic heart disease.

Summary

The scanty literature on the association of active pulmonary tuberculosis and the tetralogy of Fallot has been reviewed.

Up to January, 1959, a total of 173 patients were diagnosed in this hospital as having the tetralogy of Fallot or one of its variants. Of the 173, there were 10 patients with associated active pulmonary tuberculosis. Treatment of patients with these associated lesions needs to be individualized. After the pulmonary lesion has received the maximal benefit from chemotherapy, treatment of the cardiac lesion can be carried out with an acceptable risk. When the pulmonary lesion needs to be treated surgically, individual circumstances dictate whether the pulmonary or cardiac lesion should be corrected first. In one instance, a left upper lobectomy and simultaneous Blalock operation were carried out with success.

In none of the cases has there been evidence of extension or reactivation of the pulmonary tuberculous process following a systemic pulmonary arterial anastomosis for the tetralogy of Fallot.

Sumario

La Asistencia de la Tetralogía de Fallot cuando Está Complicada por Tuberculosis Pulmonar

Repásase la escasa literatura relativa a la asociación de tuberculosis pulmonar activa y la tetralogía de Fallot.

Hasta enero de 1959, se diagnosticó en el hospital de los AA. que un total de 173 enfermos tenía la tetralogía de Fallot o una de sus variantes. De los 173, hubo 10 que padecían de tuberculosis pulmonar activa coexistente. El tratamiento de los enfermos que tienen estas lesiones asociadas debe ser individualizado. Después que la lesión pulmonar ha recibido el beneficio máximo de la quimioterapia, puede llevarse a cabo el tratamiento de la lesión cardíaca con un riesgo aceptable. Cuando la lesión pulmonar necesita tratamiento quirúrgico, las circunstancias del caso dictan si debe rectificarse primero la lesión pulmonar o la cardíaca. En un caso, se ejecutaron con éxito una lobectomía superior izquierda y una operación de Blalock simultánea.

En algunos de los casos, ha habido signos de difusión o reactivación del proceso tuberculoso pulmonar a continuación de una anastomosis arterial pulmonar sistémica para la tetralogía de Fallot.

Resume

Conduite à tenir dans la tétrade de Fallot compliquée de tuberculose pulmonaire

Les auteurs passent en revue le peu de littérature publiée sur l'association de tuberculose pulmonaire active et de tétrade de Fallot.

Jusqu'à Janvier 1959, un total de 173 malades reçurent le diagnostic de tétrade de Fallot ou une de ses formes. Parmi les 173, il y eut dix malades porteurs d'une tuberculose pulmonaire active associée. Le traitement des malades avec des lésions associées doit être individualisé. Après que la lésion pulmonaire a retiré le maximum de bénéfice de la chimiothérapie, le traitement cardiaque peut être envisagé comme risqué mais acceptable. Quand la lésion pulmonaire doit être traitée chirurgicalement les circonstances individuelles décident laquelle de la lésion cardiaque ou de la lésion pulmonaire doit être corrigée la première. Dans un seul cas, une lobectomie du sommet gauche et une opération de Blalock furent pratiquées simultanément.

Dans aucun des cas, il n'y eut évidence d'extension ou de réactivation du processus tuberculeux après anastomose de l'artère pulmonaire pour traiter la tétrade de Fallot.

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THE USE OF INTERMITTENT POSITIVE PRESSURE BREATHING IN THE PREVENTION OF THE CARBON DIOXIDE NARCOSIS ASSOCIATED WITH OXYGEN THERAPY[1, 2]

WILLIAM FRAIMOW, RICHARD T. CATHCART, AND ELLIOTT GOODMAN

(Received for publication October 23, 1959)

Introduction

The treatment of advanced ventilatory insufficiency, regardless of its etiology, is one of the more difficult and discouraging problems in pulmonary disease. The patient is frequently apprehensive and markedly dyspneic. In addition, cyanosis as well as hypercapnea may be present. In such a situation, a therapeutic paradox may be encountered, namely, that the administration of oxygen to relieve cyanosis may result in increased carbon dioxide retention (1–6). The mechanism has usually been attributed to the removal of the hypoxic stimulus, with a resultant decrease in pulmonary ventilation. This may markedly accelerate carbon dioxide retention and lead to respiratory acidosis, coma, and sometimes death. Since this paradox has been recognized, numerous measures have been advocated for its treatment. These have included the use of oxygen-carbon dioxide mixtures, abdominal belts (7), pneumoperitoneum (8), low flows of oxygen by nasal catheter (9), bronchodilators and, more recently, mechanical breathing devices.

Hypercapnea is usually a manifestation of inadequate alveolar ventilation, even though minute volume may be adequate. Ideally, the treatment or prevention of hypercapnea is based on measures which promote ventilation. Among the mechanical measures advocated to maintain ventilation have been the Drinker respirator (10–13), the exsufflator (14), and the intermittent positive pressure breathing machine (7, 15–17). The present study was undertaken to evaluate further the effectiveness of intermittent positive pressure breathing as a means of providing hyperventilation in such a situation. In particular, it was important to determine whether the concomitant use of intermittent positive pressure breathing with oxygen could avert the effects of hypoventilation usually noted when the latter was used alone. Bronchodilators were omitted in order to delineate more fully the role of intermittent positive pressure breathing alone, unenhanced by the administration of aerosol agents.

[1] From Barton Memorial Division, Jefferson Medical College Hospital, Philadelphia, Pennsylvania.

[2] This study was supported by the Anthracite Health and Welfare Fund.

Materials and Methods

A total of 37 studies were performed upon 35 male patients with various degrees of pulmonary emphysema. Twenty-seven of these patients had associated anthracosilicosis. The patients were not being actively treated for their pulmonary emphysema at the time of the study. The severity of the emphysema was gauged by measurements of the vital capacity, maximal breathing capacity, and residual volume. The vital capacity and maximal breathing capacity were measured with a 13.5-liter Collins respirometer. The functional residual capacity was measured in duplicate by the open-circuit method (18). Arterial blood was obtained through an indwelling Cournand needle placed in the brachial artery and analyzed for oxygen content, oxygen capacity, and carbon dioxide content, using the Van Slyke and Neill manometric apparatus. Duplicates were required to check within 0.20 ml. per 100 ml. for oxygen and within 0.40 ml. per 100 ml. for carbon dioxide. Arterial pH was measured at 37°C. with a Cambridge electron-ray research model pH meter. Carbon dioxide tensions were calculated from the Henderson-Hasselbalch equation. The expired gases were collected for analysis in a 125-liter Tissot gasometer. Oxygen and carbon dioxide contents were determined by the micro Scholander technique. Duplicates were required to check within 0.03 per cent.

The subjects breathed through a mouthpiece which was attached to a three-way valve. The latter was then connected on one side to the specific gas mixture to be used and on the other to a Tissot gasometer. Initially the patients breathed 21 per cent oxygen for twenty minutes. This was delivered from an analyzed tank of compressed air. At the end of this time ventilation was measured for a three-minute period and expired gas was collected for analysis. A sample of arterial blood was drawn during the second minute. Following this the patient was given 100 per cent oxygen to breathe for twenty minutes.

TABLE 1

RESULTS OF INTERMITTENT POSITIVE PRESSURE BREATHING TESTS IN 35 PATIENTS

Patient	Maximal Breathing Capacity (Per Cent of Predicted)	Vital Capacity (Per Cent of Predicted)	RV/TLC (Per Cent of Predicted)	Index of Intrapulmonary Mixing*	Ambient Air							100 Per Cent Oxygen							Intermittent Positive Pressure Breathing with 100 Per Cent Oxygen						
					O_2 Saturation	CO_2 Volume Per Cent	pCO_2 mm. Hg	pH	Ventilation L/min/M^2	Tidal Volume cc.	CO_2 Excretion cc/min/M^2	O_2 Saturation	CO_2 Volume Per Cent	pCO_2 mm. Hg	pH	Ventilation L/min/M^2	Tidal Volume cc.	CO_2 Excretion cc/min/M^2	O_2 Saturation	CO_2 Volume Per Cent	pCO_2 mm. Hg	pH	Ventilation L/min/M^2	Tidal Volume cc.	CO_2 Excretion cc/min/M^2
1	40	51	64	8.56	86.4	66.89	74	7.31	4.71	544	113	100+	70.32	80	7.31	4.01	480	—	100+	12.68	55	7.39	—	—	—
2	55	62	62	3.35	89.5	51.37	47	7.38	5.23	318	115	100+	52.42	54	7.33	5.84	406	—	100+	48.62	41	7.41	—	—	—
3	60	69	60	5.42	92.3	53.24	43	7.44	4.07	458	114	100+	54.15	48	7.40	4.76	475	—	100+	48.62	36	7.49	—	—	—
4	—	—	—	—	95.0	51.89	45	7.41	5.25	661	129	100+	54.69	54	7.36	4.42	452	—	100+	47.28	35	7.48	—	—	—
5	138	119	34	1.85	95.8	52.54	43	7.42	3.82	683	123	100+	51.21	41	7.43	4.57	853	—	100+	36.27	15	7.70	—	—	—
6	42	73	43	3.23	90.4	57.54	50	7.41	5.32	377	124	100+	59.25	58	7.38	5.43	370	115	100+	49.23	32	7.56	—	—	—
7	61	83	59	6.37	91.1	53.41	48	7.42	6.46	424	118	100+	55.06	50	7.39	4.82	383	100	100+	44.96	21	7.58	—	—	—
8	32	46	74	6.83	88.6	57.96	51	7.40	7.69	481	121	100+	60.55	62	7.33	5.38	360	103	100+	53.88	40	7.48	—	—	—
9a†	—	—	—	—	79.1	63.87	71	7.31	5.13	347	104	100+	66.73	83	7.26	3.30	285	91	100+	60.85	57	7.39	—	—	—
9b†	—	—	—	—	84.2	63.53	67	7.33	4.48	—	111	100+	67.77	79	7.28	2.77	—	76	100+	59.68	52	7.41	—	—	—
10	33	63	63	8.03	79.0	65.18	77	7.29	4.73	—	95	91‡	64.43†	81‡	7.29‡	3.63	—	98‡	100+	61.92	67	7.35	—	—	—
11	28	47	62	2.96	89.9	57.92	53	7.39	5.47	461	117	100+	59.03	57	7.36	4.64	391	104	100+	51.51	37	7.50	—	—	—
12	94	100	55	4.78	93.4	49.64	45	7.38	6.31	469	95	100+	49.29	46	7.37	6.50	531	104	100+	48.53	45	7.37	3.55	—	102
13	48	56	49	3.65	90.1	49.88	44	7.40	5.16	460	95	100+	50.00	47	7.39	6.55	417	99	100+	45.79	34	7.48	7.22	689	125
14	21	63	75	16.99	87.4	65.72	76	7.29	4.33	311	111	100+	69.20	90	7.25	2.77	274	91	100+	66.93	69	7.34	—	—	—
15	28	51	74	7.84	90.4	53.46	45	7.42	6.82	430	—	100+	56.53	53	7.38	6.69	456	—	100+	54.17	46	7.41	7.18	644	—
16	27	66	—	—	92.1	45.87	40	7.40	6.00	502	97	100+	47.53	46	7.36	5.88	552	100	100+	42.34	32	7.47	6.60	739	122
17	45	51	60	2.14	86.6	57.44	52	7.39	3.71	343	95	100+	58.30	56	7.36	4.15	350	105	100+	51.29	33	7.53	7.70	710	138
18	50	84	44	2.98	91.9	46.23	40	7.39	4.76	458	107	100+	48.56	43	7.39	4.65	425	97	100+	40.34	31	7.45	8.49	1071	117
19	32	57	54	2.80	93.6	51.37	49	7.37	3.77	377	89	100+	49.97	48	7.36	4.48	448	104	100+	41.01	25	7.57	8.45	892	138
20	22	49	61	3.26	83.4	65.23	64	7.35	5.50	378	129	100+	66.76	67	7.34	4.20	291	98	100+	63.20	61	7.36	4.90	453	103
21	89	82	41	2.16	90.5	54.17	44	7.43	4.76	446	110	100+	57.63	52	7.38	4.19	349	85	100+	45.09	24	7.60	13.08	934	177
22	17	57	54	2.30	86.2	49.36	43	7.39	7.88	508	153	100+	50.80	51	7.33	7.09	457	126	100+	46.81	37	7.44	7.95	729	148
23	28	68	60	8.25	90.1	47.20	43	7.37	4.66	357	94	100+	48.79	45	7.36	4.48	327	92	100+	39.30	28	7.49	7.78	1082	118
24	46	58	61	4.77	92.1	51.44	45	7.40	4.04	409	98	100+	52.65	48	7.38	4.14	397	90	100+	45.61	30	7.52	6.62	1005	126
25	23	63	55	2.68	92.6	52.05	47	7.39	4.85	492	115	100+	52.90	54	7.34	4.51	437	107	100+	44.23	28	7.56	7.25	857	124
26	16	43	—	—	87.0	64.06	64	7.37	5.00	302	100	100+	68.17	84	7.26	4.30	260	97	100+	65.14	69	7.33	4.30	519	122
27	20	41	70	6.67	89.4	56.40	54	7.35	3.46	415	99	100+	59.30	62	7.31	3.18	339	92	100+	55.70	46	7.42	4.20	809	111
28	—	—	—	—	92.7	50.62	44	7.40	6.29	522	—	100+	52.08	45	7.40	6.15	510	—	100+	47.11	27	7.57	9.07	829	—
29a†	54	62	65	—	90.4	44.84	34	7.47	6.28	537	106	100+	43.70	34	7.45	7.53	676	126	100+	37.15	20	7.63	11.66	1046	136
29b†	54	62	65	—	89.2	41.62	30	7.49	8.21	789	—	100+	43.92	32	7.49	5.44	465	—	100+	40.60	27	7.54	10.11	864	—
30	29	49	60	2.07	90.1	55.70	52	7.37	3.51	409	112	100+	56.00	51	7.40	4.07	375	109	100+	51.50	40	7.47	4.68	585	107
31	23	56	65	5.59	92.0	51.82	48	7.38	6.21	568	136	100+	51.15	45	7.40	5.70	521	105	100+	47.74	34	7.49	—	—	—
32	24	62	62	1.91	92.4	50.17	40	7.43	8.95	622	162	100+	51.76	42	7.43	7.82	544	134	100+	48.54	33	7.50	11.78	688	129
33	30	54	55	10.37	91.0	55.06	51	7.38	4.09	316	115	100+	56.75	60	7.32	4.12	355	119	100+	49.54	38	7.47	—	—	—
34	51	69	53	3.67	90.5	51.85	48	7.38	4.94	636	133	100+	52.34	47	7.40	5.03	571	128	100+	46.65	36	7.46	7.37	837	136
35	26	71	71	13.36	25.1	86.21	100	7.23	5.36	284	—	95.2	94.20	147	7.09	4.33	246	—	100+	79.32	57	7.47	—	—	—
Averages					87.9	55.21	51.6	7.38	5.33	460	113.3	99.5	56.87	57.9	7.35	4.67	429	103.4	100+	50.52	38.9	7.48	7.61	799	126.6

* Per cent of alveolar nitrogen after seven minutes of 100 per cent oxygen breathing. † Studies done on the same patient on two different admissions.

‡ Breathing 40 per cent oxygen.

The ventilation was measured and arterial blood was collected at the end of the period. One hundred per cent oxygen was continued as the inspiratory gas, but was administered by intermittent positive pressure breathing[3] for the final twenty-minute period. The ventilation was again measured and arterial blood was collected and analyzed in the same fashion. Every effort was made to maintain a steady state during each breathing period. Rarely did an increase in ventilation associated with the drawing of arterial blood occur. This accounts for the occasional slight discrepancy between ventilation and arterial pressure of carbon dioxide (pCO_2) values. These were small and had no effect upon the average values.

Results

The results for the entire group are tabulated and averaged in table 1. It may be seen that the administration of 100 per cent oxygen was accompanied by a decrease in ventilation, tidal volume, and carbon dioxide excretion. In contrast to this, when 100 per cent oxygen was given by intermittent positive pressure breathing, ventilation was markedly increased, and resulted in a significant increase in carbon dioxide excretion.

The effectiveness of intermittent positive pressure breathing in preventing hypercapnea may be seen in the average values of the arterial pCO_2 for the entire group of patients following the administration of 100 per cent oxygen, and then 100 per cent oxygen and intermittent positive pressure breathing. The average pCO_2 initially had been 51.4 mm. of mercury and rose to 57.6 mm. of mercury with oxygen breathing. As a result of the increased ventilation and carbon dioxide excretion with intermittent positive pressure breathing, the average pCO_2 fell to 39.2 mm. of mercury. Not only did the pCO_2 fall, but it fell below the control value in all but 2 patients.

For purposes of analysis, the subjects were divided into groups according to the severity of their disease. One grouping was based on the initial level of the arterial pCO_2. *Group 1* represents those patients with an original pCO_2 below 45 mm. of mercury. *Group 2* is composed of patients with an arterial pCO_2 from 45–60 mm. of mercury, and *Group 3* represents those patients whose original arterial pCO_2 was more than 60 mm. of mercury.

A decrease in total ventilation was observed in all three groups when 100 per cent oxygen was administered. This was caused primarily by a decreased tidal volume which significantly affected the alveolar ventilation and resulted in a diminution of carbon dioxide excretion. These results are graphically illustrated in figure 1*A*. Since the patients were in a basal state and there was no essential alteration in oxygen consumption, variations in carbon dioxide excretion reflect changes in alveolar ventilation rather than an altered state of metabolism. The initial values for ventilation, tidal volume, and carbon dioxide excretion were lower the more severe the patients' disease. The decrease in these parameters following oxygen breathing was similarly related to the severity of the emphysema. The significance of this decrement became even more evident when percentages rather than absolute values were plotted. In *Group 1* the average decrease represented 2, 5, and 8 per cent of the

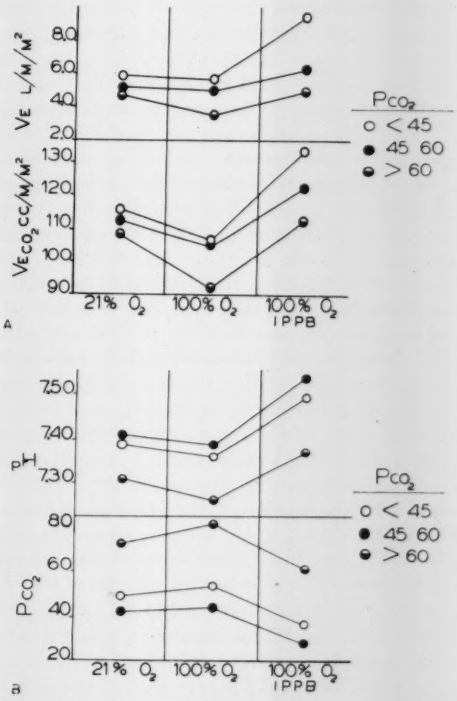

FIG. 1. Patients grouped by pCO_2. *A*. Average ventilatory changes following 100 per cent oxygen and then 100 per cent oxygen and intermittent positive pressure breathing. *B*. Average changes in arterial pCO_2 and pH.

[3] Bennett Pressure Breathing Therapy Unit, Model TV-2P.

original minute volume, tidal volume, and carbon dioxide excretion, respectively. In *Group 2* comparable declines were 5, 6, and 6 per cent, and in *Group 3* they were 25, 15, and 16 per cent. Thus, patients in *Group 3* with the most severe disease had the most significant decreases in ventilation, and these were superimposed on values for ventilation which were already inadequate.

These changes in ventilation were reflected in the arterial blood. There was an elevation of the pCO_2 in each group and a more acidotic pH (figure 1*B*). The most marked increase, as expected, occurred in *Group 3*, the pCO_2 going from 74.1 mm. to 88.9 mm. of mercury. This was accompanied by a significant pH shift.

Utilizing the same parameters, the average changes following the addition of intermittent positive pressure breathing can be summarized. There was a significant increase in minute volume, tidal volume, and carbon dioxide excretion when intermittent positive pressure breathing was used. The most marked changes occurred in *Group 1* and the least marked in *Group 3* (figure 1*A*). Here again, it may be seen that the severity of the underlying disease predetermines to some extent the degree of ventilatory increase which might be expected. Patients with the least severe emphysema were able to increase their ventilation markedly while those with more disease increased theirs to a lesser extent. Despite this, all of the patients in the more advanced group did actually increase their ventilation. The average increase in minute volume was 26 per cent in this group as compared with increments of 30 and 63 per cent in patients with less advanced disease and was accompanied by a marked increase in tidal volume (59 per cent) and a 23 per cent increase in carbon dioxide excretion.

The significance of this was clearly seen when the effects upon the arterial pCO_2 and pH were analyzed. There was a marked fall in the pCO_2 in all groups and a concomitant rise in pH. Although the percentile change in pCO_2 was similar in all three groups (39, 33, and 32 per cent, respectively), its importance is accentuated in *Group 3*, in which acidosis was most severe. In this group the pCO_2 dropped from 88.9 to 60.9 mm. of mercury. The increase in minute volume and tidal volume in this group resulted in improved alveolar ventilation and carbon dioxide excretion and caused a major drop in pCO_2.

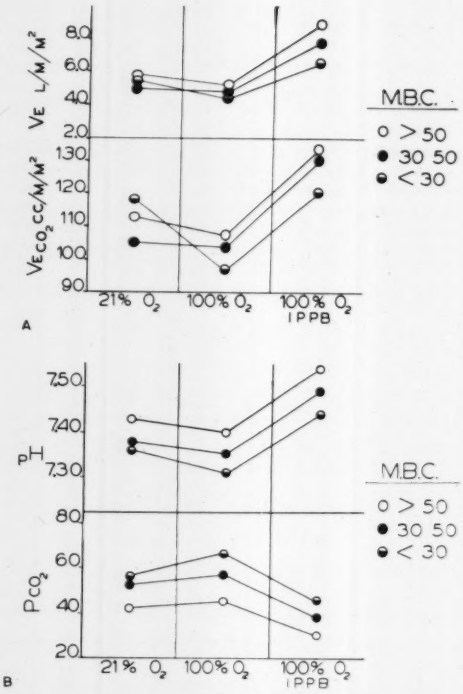

FIG. 2. Patients grouped by maximal breathing capacity. *A*. Average ventilatory changes following 100 per cent oxygen and then 100 per cent oxygen and intermittent positive pressure breathing. *B*. Average changes in arterial pCO_2 and pH.

In order to correlate more fully the ventilatory response with the severity of the disease, the patients were grouped, first, according to their ventilatory ability (maximal breathing capacity) (figure 2*A*, *B*) and, second, according to the magnitude of the residual volume to total capacity ratio (figure 3*A*, *B*). Regardless of which parameter was used, essentially the same correlation was noted. Those patients with the severest ventilatory impairment had the most significant decrease in ventilation, and resultant hypercapnea followed the administration of 100 per cent oxygen. The degree of hyperventilation achieved by intermittent positive pressure breathing was also a reflection of the severity of the patients' emphysema. In all groups, however, the pCO_2 was markedly decreased as a result of the increased carbon dioxide excretion.

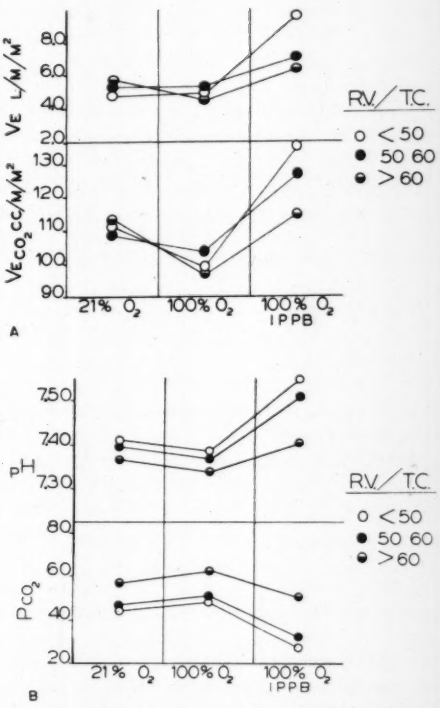

FIG. 3. Patients grouped by residual volume/total lung capacity. *A*. Average ventilatory changes following 100 per cent oxygen and then 100 per cent oxygen and intermittent positive pressure breathing. *B*. Average changes in arterial pCO_2 and pH.

DISCUSSION

The effect of 100 per cent oxygen breathing in depressing ventilation in emphysematous patients with carbon dioxide retention has been observed for some years (1–6).

The elevated carbon dioxide levels in this group of patients are a reflection of inadequate alveolar ventilation. This may be present despite a normal or above normal minute volume of ventilation. Further depression of ventilation, no matter how produced, usually results in increased hypercapnea. This may be due to increased bronchospasm, oversedation, pulmonary infection, atelectasis, or the injudicious use of oxygen.

Both inspired carbon dioxide and hypoxia act as stimuli to respiration in the normal patient. In the normal person, carbon dioxide is quite a potent stimulus. The emphysematous patient with hypercapnea, however, no longer exhibits a normal ventilatory response to increased carbon dioxide (19–22). This may be due to a depression of the responsiveness of the respiratory center to the stimulus of an increased pCO_2. It also may be due in part to the significant decrease in the patient's ventilatory capacity. This patient then is more dependent upon the effect of hypoxia upon aortic and carotid chemoreceptors acting to maintain the ventilatory drive. As the degree of hypoxia may be quite marked, this becomes a potent effect. Correction of the anoxemia by the administration of 100 per cent oxygen removes this stimulus. The result may be a further decrease in an already inadequate ventilation.

Since the paradoxical response of the cyanotic emphysematous patient to 100 per cent oxygen has been described, a number of measures have been employed to prevent it. Barach (9) believes that patients can become adapted to high oxygen tensions if the gas is administered in a stepwise fashion. He has advocated the introduction of intranasal oxygen at flow rates of 1 liter per minute, with continuation if there is no evident depression of the sensorium. Even when the oxygen is administered in this fashion, some patients have become comatose (17, 23). Although the anoxemia may be successfully corrected in this manner, little has been done to treat the hypercapnea. It is hoped to maintain the patient until other therapeutic measures, such as bronchodilators, antimicrobial drugs, and proper tracheal toilet will improve his condition.

Some measures have been directed at increasing the responsiveness of the respiratory center. The employment of oxygen-carbon dioxide mixtures (24) to accomplish this has been demonstrated to be ineffective and very dangerous (15). The use of chemical agents such as salicylates (25) and pentylenetetrazol (Metrazol®) (26) to stimulate ventilation has also been unsatisfactory in this group of patients.

One method of mechanical hyperventilation that has been employed successfully is the Drinker respirator. The maintenance of ventilation has permitted the simultaneous use of 100 per cent oxygen. In some instances in which there has been asynchronism between the patients' respiratory rate and the rate of the

respirator, adequate ventilation has not been achieved. It has been suggested that further depression of the respiratory center by opiates may allow the respirator to more effectively ventilate the patient (17). Cherniak (14) believed that more adequate ventilation could be attained by the exsufflator than with the respirator. The use of intermittent positive pressure breathing to hyperventilate these patients mechanically has been recommended (15) and successfully used in a number of cases (16, 17). It was believed that with intermittent positive pressure breathing it might be possible to administer 100 per cent oxygen without the decrease in ventilation which might otherwise occur. Of equal importance, the increased ventilation allowed a direct approach to the twin evils of hypoxia and hypercapnea.

Some writers have not shared this optimistic appraisal of the role of intermittent positive pressure breathing in the treatment of respiratory acidosis and narcosis. Cullen (23) stated that, even though hyperventilation could be attained with intermittent positive pressure breathing in many patients, it was not an effective mode of therapy. He believed its chief effect was as a means of administering bronchodilators and that any clinical improvement noted was attributable to this. The present study was undertaken to evaluate objectively how effectively and safely intermittent positive pressure breathing could be employed with 100 per cent oxygen in this clinical situation. In order not to cloud the issue, it was employed without the use of a bronchodilator.

As was expected, those patients with the least disease, as noted by any of the parameters measured, were able to be hyperventilated more effectively. Despite this, a significant increase in ventilation was achieved in even those patients with marked emphysema, whether this was judged by the maximal breathing capacity, arterial pressure of carbon dioxide, or residual volume/total lung capacity. Seventeen of 21 patients showed an increased volume of ventilation with intermittent positive pressure breathing over control values. Of further significance, 19 of 21 patients increased their minute volume when switched from 100 per cent oxygen to intermittent positive pressure breathing and 100 per cent oxygen. These changes were accompanied in most instances by a significant increase in tidal volume. What is most important clinically, of course, is that all but one of 37 patients showed a decrease in pCO_2 when intermittent positive pressure breathing was added to the 100 per cent oxygen. In addition, in only 2 patients was the final level of the pCO_2 higher than the pCO_2 measured with the patient breathing ambient air. This demonstrated conclusively that oxygen could be administered safely by intermittent positive pressure breathing to these emphysematous patients. Not only was further hypercapnea prevented, but that already present was effectively treated without the addition of bronchodilators and other therapeutic measures. This was evident even in those patients with a pCO_2 over 60 mm. of mercury or a maximal breathing capacity below 35 per cent, despite the belief of Cullen (23) that intermittent positive pressure breathing is frequently ineffective in this group.

Thus it appears that intermittent positive pressure breathing, even when used with 100 per cent oxygen, plays a role in the reduction of hypercapnea in patients with respiratory acidosis. This role is independent of the use of bronchodilators, antimicrobial drugs, tracheotomy, and other therapeutic measures. In actual clinical practice when all of the additional measures will be utilized, intermittent positive pressure breathing has an additive value and is not merely a temporizing feature as is the use of slow flows of oxygen by nasal catheter.

Summary

Thirty-five patients were studied to evaluate the role of intermittent positive pressure breathing in preventing the increasing hypercapnea associated with 100 per cent oxygen breathing in emphysematous patients.

The breathing of 100 per cent oxygen produced a decrease in ventilation accompanied by an increase in arterial pressure of carbon dioxide (pCO_2). When the 100 per cent oxygen was administered by intermittent positive pressure breathing, there was a marked increase in ventilation associated with a sharp reduction in pCO_2.

The use of intermittent positive pressure breathing to administer 100 per cent oxygen prevented the rising hypercapnea usually found in these patients when 100 per cent oxygen was administered alone. In all but 2 patients, increasing hypercapnea was not only prevented by the use of intermittent positive pressure breathing, but that already present was reduced. The

results obtained with intermittent positive pressure breathing were independent of its role as a vehicle for the administration of bronchodilators.

Sumario

El Uso de la Respiración a Presión Positiva Intermitente en la Prevención de la Narcosis por Bióxido de Carbono Asociada con la Oxigenoterapia

Se estudió a 35 enfermos para justipreciar el papel de la respiración a presión positiva intermitente en la prevención de la hipercapnea creciente asociada con respiración de oxígeno al 100 por ciento en los enfisematosos.

La respiración de oxígeno al 100 por ciento produjo una disminución de la ventilación, acompañada de aumento de la tensión arterial del bióxido de carbono (pCO_2). Cuado se administró oxígeno al 100 por ciento con la respiración de presión positiva intermitente, hubo un notable aumento de la ventilación asociado con una reducción aguda del pCO_2.

El uso de la respiración a presión positiva intermitente para administrar oxígeno al 100 por ciento impidió la hipercapnea creciente observada habitualmente en estos enfermos al administrar oxígeno al 100 por ciento por sí solo. En todos los enfermos menos 2, no tan sólo se impidió la hipercapnea creciente con el uso de la respiración a presión positiva intermitente, sino que se rebajó la que ya existía. Los resultados obtenidos con la respiración a presión positiva intermitente son independientes de su papel como vehículo para la administración de vasodilatadores.

Resume

Emploi de la respiration intermittente sous pression positive dans la prévention de l'effet narcotique du di-oxyde de carbone associé au traitement par l'oxygène

Trente cinq malades furent étudiés pour évaluer le rôle de la respiration sous pression positive dans la prévention de l'hypercapnée qui se produit lorsqu'on y associe 100% d'oxygène chez les malades emphysèmateux.

La respiration de 100% d'oxygène produit une diminution de la ventilation ainsi qu'une augmentation de la pression artérielle du gaz carbonique (pCO_2). Lorsqu'on administra 100% d'oxygène d'une façon intermittente sous pression positive, il se produisit une augmentation marquée de la ventilation, associée à une réduction notable de pCO_2.

L'emploi de la respiration intermittente sous pression positive pour donner 100% d'oxygène prévint l'augmentation de l'hypercapnée habituellement trouvée chez ces malades, quand on leur donne seulement 100% d'oxygène. Chez tous les malades, sauf deux, l'augmentation de l'hypercapnée ne fut pas seulement prévenue par l'inhalation intermittente positive, mais il y eut aussi une réduction de l'hypercapnée présente. Les résultats obtenus par l'inhalation intermittente sous pression positive furent indépendants de son rôle de véhicule dans l'administration de bronchodilatateurs.

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THE EFFECT OF HELIUM AND OXYGEN MIXTURES ON PULMONARY RESISTANCES IN EMPHYSEMA[1, 2]

BIRGER GRAPÉ,[3] ELIHU CHANNIN, AND JOHN M. TYLER

(Received for publication October 28, 1959)

INTRODUCTION

Helium is considerably less dense than air, and this property has led to the belief that a mixture of 80 per cent helium and 20 per cent oxygen would increase the ease of gas flow within the lungs. Therefore, this mixture has been advocated for use as inspired gas in the treatment of patients with obstructive disease of the lungs. In 1934, Barach (1) reported symptomatic improvement in asthmatic subjects breathing helium and oxygen. He subsequently demonstrated (2) that this mixture decreased the resistance to the flow of gas through a constricting orifice placed at the mouth, and he believed that this explained the improvement found in his patients. More recent investigation on the effect of gas mixtures such as this have been limited to studies of the flow resistance of the lungs of experimental animals, normal subjects, and a few patients with asthma (3–5). There have been no measurements of its effect on the resistance to gas flow in patients with severe emphysema; accordingly, an investigation has been made of the effect of 80 per cent helium and 20 per cent oxygen on pulmonary resistance and compliance in 10 patients with severe obstructive disease of the lungs.

METHODS

The patients selected had emphysema diagnosed on the basis of typical history, physical and laboratory examination, and pulmonary function studies. They were all studied after they had been in the hospital for at least two weeks and were receiving intensive therapy, including bronchodilators, during this period. In spite of this therapy, they all had persistence of symptoms, particularly dyspnea. Vital capacity with its one- and two-second fractions and maximal breathing capacity were obtained by use of a Collins respirometer. Total lung capacity, with its subdivisions, was estimated by a closed-circuit helium technique as described by Gilson and Hugh-Jones (6). Duplicate studies agree within 10 per cent.

The closed-circuit breathing apparatus used is illustrated in figure 1. The seated patient breathed through a pneumotachograph and the flow recording was used to indicate the point of zero flow. Tidal volume was obtained both graphically from the spirometer and electrically from a potentiometer attached to the spirometer wheel. The compliance was then estimated from end-inspiratory and end-expiratory pressure at these zero flow points and the volume change.

A 15-cm. esophageal balloon-catheter was used to obtain an approximate intrapleural pressure, as used by Mead and his associates (7). A low internal volume, minimal displacement differential transducer[4] was used for the pressure determination, and all recordings were made with a recording oscillograph.[5] The patient's oxygen consumption was balanced by adding oxygen so as to keep the apparatus volume constant. Total resistances were calculated by the method of Frank and co-workers (8), using a mean flow and mean pressure. Inspiratory and expiratory resistances were calculated by obtaining the flow rate from a tangent drawn to the volume curve at approximately midvolume; then, assuming that the relationship of the volume change to the pressure necessary to overcome elastic forces was linear, pressure in excess of this was used to overcome resistance. In addition, resting end-tidal transpulmonary pressures were obtained by clamping the tube between the pneumotachograph and the closed-circuit apparatus after the patient had completed a quiet expiration. Maximal static transpulmonary pressures were obtained at the peak of inspiration. Static compliance was determined by stepwise interruption of a maximal inspiration, interruption being obtained by clamping the tube leading from the pneumotachograph to the closed-circuit apparatus. The compliance was then estimated as the slope of the line in the tidal range, as obtained by plotting the transpulmonary pressure at each volume

[1] From the Pulmonary Laboratory and the Medical Service of the Lemuel Shattuck Hospital, Jamaica Plain, Massachusetts; the Department of Physiology, Harvard School of Public Health, Boston, Massachusetts; and the Tufts University School of Medicine, Medford, Massachusetts.

[2] Supported by a grant-in-aid from the American Trudeau Society.

[3] Lederle Fellow. Present address: St. Görans Hospital, Stockholm, Sweden.

[4] Sanborn Model 267 B Pressure Transducer, Sanborn Company, Waltham, Massachusetts.

[5] Sanborn Polyviso, Sanborn Company, Waltham, Massachusetts.

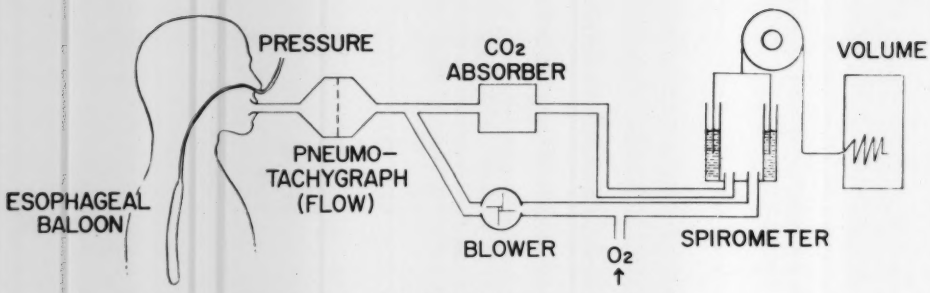

FIG. 1. Diagram of the closed circuit apparatus employed.

step. Measurements of dynamic compliance, inspiratory, expiratory, and total pulmonary resistances were made at a normal breathing rate and again at approximately twice this rate. All measurements were then repeated after equilibration with 80 per cent helium and 20 per cent oxygen for fifteen minutes.

RESULTS

Clinical information on the patients studied is provided in table 1. The measurements of vital capacity and the timed fractions, the maximal breathing capacity, and the total lung capacity with its subdivisions obtained in the 10 patients are given in table 2. The observations of the change produced by helium and oxygen in the functional residual capacity at the two respiratory rates, transpulmonary pressures, and static compliance are given in table 3. The alteration in dynamic compliance produced by the helium mixture is given in table 4.

The pulmonary resistances obtained with air and the percentage of change produced by helium and oxygen are given in table 5. There was a decrease of 22.0 ± 2.8 per cent (±1 standard error) from the values obtained with air in the total resistance during slow breathing, and 17.8 ± 5.2 per cent during rapid breathing. Changes in inspiratory resistances showed a fall of 24.7 ± 4.2 per cent on slow breathing, and 24.3 ± 4.3 per cent on rapid breathing. Expiratory resistances showed a 20 ± 3.6 per cent drop during slow breathing, and a 6.5 ± 9.4 per cent drop during rapid breathing. Except for expiratory resistance at rapid respiratory rates, all of these changes are significant.

The difference in dynamic compliance between values obtained with air and the helium-oxygen mixture was +0.002 ± 0.03 L. per cm. of water during slow breathing and −0.009 ± 0.06 L. per cm. of water during the rapid rate.

All of the patients except *No. 6* showed the expected fall in dynamic compliance with rapid respiratory rates. No explanation could be found for the increase in compliance shown by this patient.

The change produced by helium-oxygen in end-expiratory pressure and maximal static transpulmonary pressure was −0.7 ± 1.1 cm. of water, and −0.15 ± 0.5 cm. of water, respectively.

The change in functional residual capacity produced by helium was −0.12 ± 0.08 L. at the slow rate, and −0.10 ± 0.05 L. at the rapid rate. The increase in functional residual capacity during air breathing brought about by the rapid respiratory rate was +0.53 ± 0.05 L., and during helium breathing it was +0.48 ± 0.06 L. The changes in compliance, static transpulmonary pressures, and functional residual capacity due to helium and oxygen are not statistically significant.

TABLE 1
CLINICAL INFORMATION ON PATIENTS STUDIED

Patient	Sex	Age	Height	Weight	Body Surface Area
			cm.	*lbs.*	*sq. meters*
1	F	63	162	87	1.35
2	M	52	173	139	1.74
3	M	48	166	98	1.45
4	M	54	161	105	1.47
5	M	35	183	120	1.70
6	M	60	183	112	1.65
7	M	73	162	129½	1.61
8	F	41	162	139	1.66
9	M	55	179	170	1.94
10	M	42	180	124	1.71

TABLE 2

ROUTINE VENTILOMETRY AND LUNG VOLUME MEASUREMENTS

Patient	Vital Capacity (Liters)					MBC*		TLC†	FRC‡	RV§	RV/TLC‖
	Total	Per Cent Predicted	Per Cent of Total			Liters	Per Cent Predicted				
			1 Second	2 Seconds	3 Seconds						
								(*L.*)	(*L.*)	(*L.*)	
1	1.17	44	54	77	88	¶		5.96	5.13	4.98	0.84
2	3.39	85	32	51	56	25	22	9.64	7.00	6.04	0.63
3	1.80	49	42	57	67	18	20	9.00	7.80	6.74	0.75
4	1.74	48	28	41	61	25	26	8.15	7.03	5.76	0.71
5	2.25	49	34	48	60	27	24	7.52	6.15	4.22	0.56
6	2.02	51	82	100	—	70	76	7.75	6.17	5.40	0.70
7	1.32	40	31	45	59	¶		6.30	5.63	5.18	0.82
8	3.33	111	59	85	91	71	83	4.92	3.10	2.13	0.43
9	2.72	67	39	55	64	42	37	6.64	5.20	3.75	0.56
10	1.83	42	44	57	68	30	27	7.29	6.01	4.99	0.59

* MBC—maximal breathing capacity.
† TLC—total lung capacity.
‡ FRC—functional residual capacity.
§ RV—residual volume.
‖ RV/TLC—residual volume/total lung capacity.
¶ Unable to perform test.

TABLE 3

CHANGES IN FUNCTIONAL RESIDUAL CAPACITY, TRANSPULMONARY PRESSURES, AND STATIC COMPLIANCE

Patient	FRC* (L.) Slow		FRC* (L.) Rapid		Static Transpulmonary Pressure							
	Air	$He\text{-}O_2$†	Air	$He\text{-}O_2$†	Change in FRC* (L.) from Slow to Rapid		End Expiratory Pressure *cm.* H_2O		Maximal Inspiratory Pressure *cm.* H_2O		Static Compliance *L./cm.* H_2O	
					Air	$He\text{-}O_2$†	Air	$He\text{-}O_2$†	Air	$He\text{-}O_2$†	Air	$He\text{-}O_2$†
1	5.08		5.43		0.35		−5.1	+6.2			0.080	
1‡	5.13	−0.07	5.50	−0.04	0.37	0.40	−0.5	−0.2	7.0	−0.5	0.120	0.000
2	7.00	+0.03	8.87	+0.19	1.87	2.03	−4.9	−1.2	12.9	−3.9	0.258	0.149
3	7.80	0.00	8.30	−0.10	0.50	0.43						
4	7.03	−0.80	7.63	−0.34	0.60	0.34	−3.4	−1.9	8.1	−3.2	0.268	0.102
5	6.15	+0.15	6.57	0.00	0.42	0.27	−5.1	+1.0	20.0	−4.0	0.098	−0.003
6	6.17	+0.07	6.55	−0.10	0.38	0.21	−3.7	+2.3	10.7	+1.4	0.148	0.011
7	5.63	−0.13										
8	3.10	−0.08	3.10	−0.14	0.00	−0.06	−1.5	−3.4	16.8	+8.2	0.108	0.014
9	5.20	−0.13	5.70	−0.18	0.50	0.45	−6.4	−4.3	11.7	−1.2	0.185	0.035
10	6.21	−0.20	6.54	−0.21	0.33	0.32	−6.3	0.0	14.7	+2.0	0.112	0.006
Mean		−0.12		−0.10	0.53	0.48		−0.17		−0.15		+0.039
±1 S.E.§		±0.08		±0.05	±0.05	±0.06		±1.1		±0.5		±0.20
P		>0.15		= 0.08		>0.90		>0.80		>0.70		>0.09
		<0.19						<0.90		<0.80		<0.11

* FRC—functional residual capacity.
† Difference from air value produced by 80 per cent helium and 20 per cent oxygen.
‡ Patient studied twice.
§ S.E.—standard error.

TABLE 4

CHANGES IN DYNAMIC COMPLIANCE PRODUCED BY HELIUM AND OXYGEN

Patient	Compliance Slow Respiratory Rate *L./cm. H_2O*		Compliance Rapid Respiratory Rate *L./cm. H_2O*	
	Air	He-O_2*	Air	He-O_2*
1	0.124	−0.030	0.055	+0.030
1†	0.099	0.000	0.043	+0.003
2	0.291	+0.069	0.136	−0.040
3	0.154	+0.005	0.067	+0.17
4	0.147	+0.009	0.119	−0.002
5	0.067	+0.011	0.035	−0.001
6	0.194	−0.023	0.337	−0.172
7	0.142	−0.021	0.055	+0.032
8	0.095	+0.006	0.110	−0.006
9	0.121	−0.022	0.084	+0.008
10	0.119	+0.023	0.067	+0.030
Mean		+0.002		−0.009
±1 S.E.‡		±0.03		±0.06
P		<0.90		>0.80 <0.90

* Difference from air value produced by 80 per cent helium and 20 per cent oxygen.

† Patient studied twice.

‡ S.E.—standard error.

The changes in resistance are also shown in figures 2 to 4. Each point represents a paired observation in the same patient, one on helium and one on air. The 45° line, therefore, represents the position at which the point would fall if the gas mixture produced no change. Thus, if a decrease in resistance was obtained when the subject breathed helium and oxygen, the paired value would be above the 45° line. In all instances, except the expiratory resistance during rapid breathing rates, the helium mixture caused a decrease in resistance.

DISCUSSION

Although it is widely known that the relative density of helium in relation to air is quite low (0.16), it is not generally appreciated that its relative viscosity is more than that of air (1.08). Theoretical analysis of the changes to be expected from the use of a gas which is less dense but slightly more viscous than air is a complicated problem.

In 1915, Rohrer (9) proposed the equation, $P = K_1\dot{V} + K_2\dot{V}^2$ when $\dot{V}$ is the rate of gas flow, P is the resistive pressure drop, and K_1 and K_2 are constants. The expression $K_1\dot{V}$ relates to the pressure necessary to overcome resistance to laminar flow, and the constant K_1 includes the effect of geometry of the respiratory tract and the viscosity of the gas. $K_2\dot{V}^2$ accounts for the pressure drop due to turbulent flow and varies with the gas density and the bend geometry of the system. McIlroy and his associates (5) have pointed out that, since helium is slightly more viscous and considerably less dense than air, one would therefore expect a small rise in the resistance to laminar flow and a considerable drop in the resistance to turbulent flow. But, as is discussed by these writers, the situation is more complicated. The proportion of laminar to turbulent flow must be the same for each gas mixture if changes in laminar and turbulent resistances are to be estimated solely on the basis of viscosity and density differences between these mixtures. This proportion of laminar to turbulent flow is related to the ratio of the viscosity to the density, i.e., the kinematic viscosity. Since helium has a kinematic viscosity of 6.75, it would markedly alter the proportion of laminar to turbulent flow, and the over-all influence of breathing this gas would be impossible to predict on theoretical grounds without detailed information as to the geometry of the air passages.

In 1941, Dean and Visscher (3) were unable to show a drop in pulmonary resistance in the experimental animal when helium was substituted for air as the inspired gas. However, as has been pointed out by McIlroy and colleagues (5), it is probable that the method they used measured the effects of hysteresis due to surface tension, which may have accounted for a large part of what they measured as flow resistance.

Using different techniques, Otis and Bembower (4) studied the effects of 80 per cent helium and 20 per cent oxygen in twenty normal subjects, and McIlroy and co-workers (5) studied the effects of this gas in two normal subjects and two patients with asthma. Both groups found decreases of 20 to 35 per cent in resistance in the subjects studied. Thus, there is evidence that helium and oxygen lower pulmonary resistance in normal subjects and in asthmatic patients. However, Schiller and his associates (10), using changes in the timed vital capacity as an index of improvement in severely ill asthmatic patients, were unable to find any change produced by helium and oxygen. In a

TABLE 5

CHANGES IN RESISTANCE PRODUCED BY HELIUM AND OXYGEN

Patient	Respiratory Rate				Total Resistance cm. H_2O/L./sec.				Inspiratory Resistance cm. H_2O/L./sec.				Expiratory Resistance cm. H_2O/L./sec.			
	Slow		Rapid		Slow		Rapid		Slow		Rapid		Slow		Rapid	
	Air	He-O_2	Air	He-O_2	Air	He-O_2*	Air	He-O_2*	Air	He-O_2*	Air	He-O_2*	Air	He O_2*	Air	He-O_2*
1	30	32	120	35	8.8	−30%	15.7	−48%	8.1	−28%	14.4	−54%	10.0	−30%	17.5	−34%
1†	22	28	70	64	13.5	−25%	11.8	−5%	13.2	−32%	10.1	−17%	14.1	−14%	14.4	+15%
2	13	14	44	73	7.5	−9%	7.7	−9%	4.8	+2%	5.7	−11%	12.5	−9%	11.1	−14%
3	23	26	40	40	10.5	−14%	11.6	+2%	9.5	−31%	10.4	−23%	12.8	−2%	13.3	+55%
4	22	21	30	38	11.8	−16%	15.2	−15%	7.5	−15%	8.6	−21%	21.5	−36%	27.7	−11%
5	19	22	54	61	16.9	−30%	17.7	−15%	12.4	−27%	10.6	−17%	26.8	−36%	34.5	−19%
6	21	26	49	31	4.9	−29%	5.4	−41%	4.3	−44%	5.0	−48%	5.6	−11%	5.8	−36%
7	21	19	42	46	15.1	−11%	17.2	+8%	14.0	−9%	14.2	−11%	19.2	−16%	24.9	+42%
8	17	18	26	31	6.3	−30%	6.8	−24%	5.8	−36%	6.4	−25%	6.9	−26%	7.4	−27%
9	20	25	43	36	5.0	−14%	6.7	−18%	4.3	−14%	5.2	−15%	5.9	−10%	8.7	−7%
10	11	11	28	38	10.3	−34%	6.5	−31%	8.5	−38%	5.2	−25%	12.9	−31%	8.1	−35%
Mean						−22%		−17.8%		−24.7%		−24.3%		−20.1%		−6.5%
±1 S.E.‡						±2.8		±5.2		±4.2		±4.3		±3.6		±9.4
P						<0.01		<0.01		<0.01		<0.01		<0.01		<0.6

* Change produced by 80 per cent helium and 20 per cent oxygen expressed in per cent of air value

† Patient studied twice

‡ S.E.—standard error

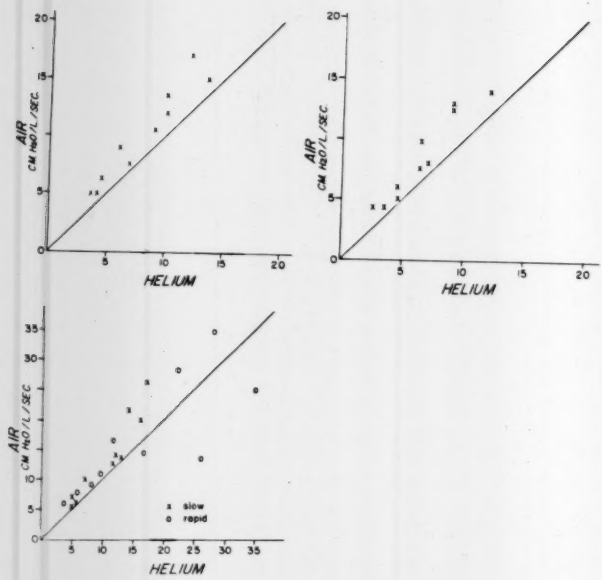

FIG. 2. (Top left). Changes in total resistance during normal respiratory rates produced by the 80 per cent helium and 20 per cent oxygen mixture. The total resistance while breathing air is plotted against that while breathing 80 per cent helium and 20 per cent oxygen in each subject. Values above the 45° line represent decreases in total resistance due to 80 per cent helium and 20 per cent oxygen.

FIG. 3. (Top right). Changes in inspiratory resistance during normal respiratory rates produced by 80 per cent helium and 20 per cent oxygen. The values are plotted in the same manner as in figure 2.

FIG. 4. (Bottom). Changes in expiratory resistance produced by 80 per cent helium and 20 per cent oxygen. Values are plotted in the same manner as in figure 2.

×—resistance during normal respiratory rates.
○—resistance during rapid respiratory rates.

few less disabled patients, slightly larger values were obtained with the helium and oxygen mixture. The present finding that the expiratory resistance during rapid respiratory rates was not significantly decreased would tend to support the findings of Schiller.

In interpreting the changes caused by inspired gases which differ from air, it is important to make comparisons between them at the same lung volume, since changes in functional residual capacity are known to alter both resistance to gas flow (11) and dynamic compliance (12). In the present study there was no significant change in functional residual capacity, so the decrease in resistance can be ascribed to the characteristic density and viscosity of the helium and oxygen mixture.

Although only a limited number of measurements was made, it is not surprising that no change in the static compliance or in the end-tidal transpulmonary pressure was produced by the helium-oxygen mixture, since the functional residual capacity did not change and helium by itself would not be expected to alter lung tissue or surface tension within the lungs. In emphysema, increasing the respiratory rate probably alters the distribution of inspired gas so that more of the tidal volume enters low resistance pathways, causing a change in dynamic compliance (13). Since helium, with its kinematic viscosity greater than air, would be expected to alter distribution of inspired gas, it might be anticipated that the decrease in dynamic compliance produced by the rapid rate would not be the same with air as it is with helium and oxygen. In the present study no difference was found, but the method might not have been sufficiently sensitive to determine

whether helium could cause the same degree of alteration in distribution at each respiratory rate.

Although it is believed that the decline in pulmonary resistances produced by helium and oxygen is therapeutically effective in the short-term treatment of severe emphysema, it must be stressed that no measurements of arterial carbon dioxide tension, oxygen consumption, or carbon dioxide production were made to permit estimation of whether ventilation was more effective.

Summary

It has been shown that, when subjects with severe emphysema breathed a mixture of 80 per cent helium and 20 per cent oxygen, there was no change in static or dynamic compliance of the lungs, functional residual capacity, or static transpulmonary pressures. There were significant decreases in pulmonary resistance during normal breathing which amounted to approximately 20 per cent of the value obtained with air. It is believed that this change in resistance is therapeutically significant in the short-term treatment of patients with severe obstructive disease of the lungs.

Sumario

El Efecto de las Mezclas de Helio y Oxígeno sobre la Resistencia Pulmonar al Enfisema

Queda demostrado que, cuando los sujetos que tenían enfisema grave respiraban una mezcla de 80 por ciento de helio y 20 por ciento de oxígeno, no había alteración de la subordinación estática o dinámica de los pulmones, la capacidad residual funcional o las presiones transpulmonares estáticas. Hubo disminuciones importantes de la resistencia pulmonar durante la respiración normal, que representaban aproximadamente 20 por ciento del valor obtenido con el aire. Parece que esta modificación de la resistencia es terapéuticamente importante en el tratamiento a breve plazo de los sujetos que padecen de grave enfermedad oclusiva de los pulmones.

Resume

L'effet du mélange helium-oxygène sur les résistances pulmonaires dans l'emphysème

On a montré que, lorsque des sujets ayant un emphysème grave respirent un mélange de 80% d'helium et 20% d'oxygène, il n'y avait pas de changement dans l'extensibilité statique ou dynamique des poumons, ni dans la capacité fonctionnelle résiduelle ou dans les pressions statiques transpulmonaires. Il se produisit dans la résistance pulmonaire, pendant la respiration normale, des diminutions notables qui atteignirent à peu près 20% de la valeur obtenue avec de l'air. On croit que ce changement de résistance a une signification thérapeutique dans le traitement à court terme de malades ayant une forme sévère d'obstruction pulmonaire.

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THE EFFECTS OF THORACIC SURGERY ON THE PULMONARY DIFFUSING CAPACITY[1, 2, 3]

FRANCES DIETIKER, WILLIAM LESTER, AND BENJAMIN BURROWS

(Received for publication November 9, 1959)

Introduction

During the past decade there have been numerous studies to determine the physiologic changes which are induced by thoracic surgery. However, little is known of the effects of operative procedures on the pulmonary diffusing capacity (D_L). It has been reported that there is some decrease in D_L in the postoperative period and that this decrease is roughly proportional to the loss of lung volume, at least following pneumonectomy (1–4). However, available studies are limited either by the absence of preoperative data, the performance of determinations only in the late postoperative period, or the failure to obtain comparable pre- and postoperative conditions. To the writers' knowledge, there are no published data concerning the changes in the pulmonary diffusing capacity induced by surgical procedures on the lung of smaller extent than pneumonectomy.

For these reasons it was decided to study the pulmonary diffusing capacity pre- and postoperatively in a series of patients subjected to various thoracic surgical procedures. In this report, data on 39 patients are presented.

Materials and Methods

The subjects comprised 35 consecutive surgical cases from the Suburban Cook County Tuberculosis Hospital-Sanitarium and 4 cases of pneumonectomy from the University of Chicago Clinics. There were 27 males and 12 females in the group, ranging in age from nineteen to sixty-seven years. Twelve patients underwent extraperiostial paraffin plombage thoracoplasties. All of the remaining 27 patients underwent pulmonary resection. These procedures consisted of 4 wedge resections, 5 pneumonectomies, and 18 lobectomies. (Included among the lobectomies is one patient who had two segments and a wedge removed, and another patient who had one lobe and one segment removed.) The average age of the plombage patients was fifty years, and that of the resection group was forty years.

All of the plombage patients had moderately advanced or far advanced pulmonary tuberculosis, and emphysema was also diagnosed in 2. Eighteen of the 27 patients with resections had tuberculosis. In 6 the tuberculosis was minimal, in 7 it was moderately advanced, and in 5 it was far advanced. Seven of the patients with resections had neoplasms, one had chronic lung abscess, and one had broncholithiasis. In addition to the above diagnoses, emphysema was diagnosed in 2 and asthmatic bronchitis in 2 of the patients who had resections.

Routine pulmonary function tests and diffusing capacities were obtained pre- and postoperatively on all subjects. Early postoperative tests were obtained before the ninth postoperative week, with an average time of five and one-half weeks for the plombage group and four weeks for the resection patients. Additional postoperative tests were obtained in 9 plombage and 6 resection patients eight to twenty-nine weeks postoperatively. The average postoperative time for these later tests was fifteen weeks for the thoracoplasty group and thirteen weeks for the resection group.

The residual volume was determined by a modification of the open-circuit method of Darling and associates (5), and the pulmonary diffusing capacity by Forster and co-workers' modification of Krogh's carbon monoxide breath-holding technique (6). Reported lung volumes were obtained by adding the previously determined residual volume to the measured inspiration of the carbon monoxide mixture at the time of breath-holding, the patients having exhaled fully before inspiring the mixture. Reported diffusing capacities represent an average of at least two determinations in which the pulmonary diffusing capacity (D_L) was corrected to the highest volume performed on that day. This was done by replacing the poorer inspired volumes with the best inspired volume before calculating the D_L. This compensates for the poorer inspiratory volumes of duplicate determinations and, therefore, gives a more accurate comparison of pre- and postoperative volumes. In one case, the breath-holding volumes of duplicate determinations differed by 700 cc. However, in most patients the determinations agreed within 150 cc. Therefore, correcting the D_L from the lower volume to the higher

[1] From the Suburban Cook County Tuberculosis Hospital-Sanitarium, Hinsdale, Illinois; and the Department of Medicine, University of Chicago, Chicago 37, Illinois.

[2] Aided by a research grant from the Illinois Tuberculosis Association.

[3] Presented before the Medical Session, as part of *Section 4A*, at the annual meeting of the American Trudeau Society, Los Angeles, California, May 17, 1960.

TABLE 1

PREOPERATIVE AND POSTOPERATIVE DATA ON PATIENTS UNDERGOING WAX PACK THORACOPLASTY

Preoperative Data						Postoperative Data				
Case Number	Sex	Age	Diagnosis	V_L*	D_L†	Weeks after Surgery	V_L	D_L	Per Cent of Change	
									V_L	D_L
1	M	60	F.A.,‡ emphysema	3,650	14.1	8	3,040	7.9	−17	−44
						29	2,930	9.7	−20	−31
2	F	33	F.A.	2,130	11.1	9	1,980	9.4	−11	−16
3	M	54	M.A.§	5,220	14.0	4	4,380	7.4	−16	−47
						12	4,470	10.5	−14	−25
						19	4,340	9.4	−17	−33
4	M	59	F.A.	3,585	11.7	5	3,140	8.6	−12	−27
5	M	55	F.A., emphysema	5,370	25.1	7	4,380	15.0	−18	−40
						15	4,765	26.9	−11	+7
						20	4,990	23.3	−7	−7
6	M	60	F.A.	5,000	16.2	3½	4,290	12.9	−14	−20
						11	4,770	16.7	−5	+3
7	M	54	F.A.	5,620	18.1	3	4,300	12.4	−23	−32
						8	4,620	13.1	−18	−28
8	F	22	F.A.	1,980	12.2	4	1,720	9.3	−13	−24
						10	1,855	10.7	−6	−12
9	M	45	F.A., bronchopleural fistula	3,760	19.7	3	2,620	12.0	−30	−39
						14	2,930	14.5	−22	−27
10	M	60	M.A.	5,160	24.1	5	3,520	12.3	−32	−49
						20	3,575	14.4	−31	−40
11	M	30	F.A.	4,520	19.9	5	4,140	21.6	−8	+8
						13	4,300	20.6	−5	+3
12	M	65	F.A.	4,520	14.9	9	3,620	11.4	−20	−24
Average of first postoperative test		50		4,210	16.8	5½	3,426	11.7	−19	−30

* V_L is the volume of the lung in cubic centimeters of dry gas at 0° and 760 mm. at the time of breath-holding.

† D_L is the diffusing capacity of the lung for carbon monoxide, and is reported in cubic centimeters of carbon monoxide diffusing into the blood per minute per mm. Hg difference between alveolar carbon monoxide tension and blood carbon monoxide tension.

‡ F.A.—far advanced tuberculosis.

§ M.A.—moderately advanced tuberculosis.

volume actually made little difference in the calculated D_L. The maximal variation of corrected D_L between duplicate determinations was 18 per cent, with an average variation of 5 per cent.

RESULTS

The determinations in all of the 39 subjects are summarized in tables 1 to 4. Thirty-six of the patients showed an initial decrease of both breath-holding lung volume (V_L) and diffusing capacity (D_L) with surgery. Two patients showed improved D_L after surgery, despite smaller volumes, and one patient showed an improved breath-holding volume postoperatively because of poor preoperative performance. His total lung capacity determined separately did show a decrease with surgery.

Twenty-nine of the patients showed a greater decrease in percentage of D_L than of V_L, 4 showed essentially the same decrease of both, and 6 patients showed a greater decrease of V_L than D_L (including the 2 patients who improved their D_L). These results are depicted in figure 1. For the group of 12 patients with wax-pack thoracoplasty, the mean initial loss of volume with surgery was 19 per cent, and the mean loss of diffusing capacity was 30 per cent. For the 18 lobectomy patients, the mean loss of volume was 26 per cent, and the mean loss of diffusing capacity was 30 per cent; for the 5

TABLE 2

PREOPERATIVE AND POSTOPERATIVE DATA ON PATIENTS UNDERGOING WEDGE RESECTION

Case Number	Sex	Age	Diagnosis	Preoperative Data V_L*	Preoperative Data D_L†	Postoperative Data Weeks after Surgery	Postoperative Data V_L	Postoperative Data D_L	Per Cent of Change V_L	Per Cent of Change D_L
13	M	35	Min.‡	4,710	18.2	6	4,220	13.9	−10	−24
						24	4,500	17.2	−5	−6
14	M	29	Min.	3,260	15.6	8	3,315	15.2	+2	−3
15	F	26	M.A.§	2,470	13.9	5	2,115	10.6	−14	−24
16	F	19	Min.	3,310	16.8	2½	2,860	12.0	−14	−29
Average of first postoperative test		27		3,438	16.1	5½	3,127	12.9	−9	−20

* V_L is the volume of the lung in cubic centimeters of dry gas at 0° and 760 mm. at the time of breath-holding.

† D_L is the diffusing capacity of the lung for carbon monoxide, and is reported in cubic centimeters of carbon monoxide diffusing into the blood per minute per mm. Hg difference between alveolar carbon monoxide tension and blood carbon monoxide tension.

‡ Min.—minimal tuberculosis.

§ M.A.—moderately advanced tuberculosis.

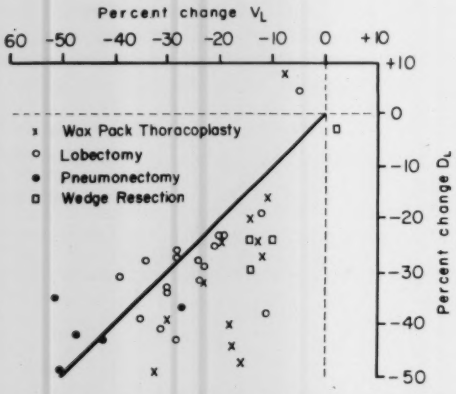

FIG. 1. Percentage of change in diffusion versus percentage of change in volume in the early postoperative period; total group of 39 patients.

pneumonectomy patients, the volume loss was 44 per cent and diffusion loss was 41 per cent; and for the 4 wedge resection patients, the mean volume loss was 9 per cent and the diffusion loss was 20 per cent.

Nitrogen washout curves remained essentially unchanged in all but 2 patients. Both of the last-named patients had abnormal washout curves before surgery which became slightly more abnormal postoperatively. However, their D_L and V_L decreases following surgery paralleled those of the other patients.

In 15 patients, tests were repeated four to twenty-one weeks after initial postoperative studies. Only 2 patients had lower D_L than on the first postoperative test, and 13 showed good improvement. Nine of these cases were post-thoracoplasty patients and had revealed a decline of 34 per cent in D_L and of 19 per cent in V_L in the early postoperative period. Subsequent tests showed a return toward preoperative values, the final D_L falling only 17 per cent and the final V_L only 14 per cent below preoperative values. The 6 resection patients (5 lobectomies and one wedge) showed a mean lung volume loss of 15 per cent and a diffusing capacity loss of 17 per cent in the late postoperative period; these same subjects had revealed a 25 per cent decline in V_L and a 32 per cent decline in D_L in their early postoperative course. These results are depicted in figures 2*A* and *B*, in which the changes in D_L and V_L are plotted against the time elapsed after surgery. In figure 2*C*, the changes in D_L are "corrected" for the alterations in V_L which occurred with surgery; it is apparent that the disproportionate fall in D_L in the early postoperative period is less apparent after the second postoperative month. These results are summarized in table 5.

The mean breath-holding volume (at standard

TABLE 3

PREOPERATIVE AND POSTOPERATIVE DATA ON PATIENTS UNDERGOING LOBECTOMY

Preoperative Data						Postoperative Data				
Case Number	Sex	Age	Diagnosis	V_L*	D_L†	Weeks after Surgery	V_L	D_L	Per Cent of Change	
									V_L	D_L
17	M	29	Min.‡	4,540	27.8	5½	3,170	18.4	−30	−34
						16	3,950	25.8	−13	−7
18	F	46	Min.	3,380	14.8	3	2,615	10.1	−23	−32
						7	2,640	10.4	−22	−30
						19	2,910	13.7	−14	−7
19	M	56	Neoplasm, emphysema	5,490	23.0	2½	3,945	13.2	−28	−43
20	F	48	Collapsed midlobe broncholithiasis	2,630	10.4	3½	2,490	10.8	−5	+4
21	M	29	F.A.§	3,610	21.5	3½	2,540	14.5	−30	−33
						10	3,210	18.5	−11	−14
22	M	37	M.A.‖	5,360	25.7	4	3,680	15.1	−31	−41
						8	4,280	19.4	−20	−25
23	M	33	M.A.	5,200	25.2	3½	3,760	18.3	−28	−27
						9	4,100	17.2	−21	−32
24	F	36	M.A.	3,160	16.2	7	2,390	11.7	−24	−28
25	F	26	M.A.	2,825	18.4	4	2,490	14.9	−12	−19
26	M	62	Neoplasm	4,510	13.6	4	3,470	9.7	−23	−29
27	M	43	M.A.	4,760	20.1	3½	3,420	14.8	−28	−26
28	F	43	F.A., asthma	3,055	9.4	4	2,490	7.2	−19	−23
29	F	44	Min.	3,560	12.6	4½	3,160	7.8	−11	−38
30	F	34	Lung abscess	4,310	25.7	4	2,850	18.5	−34	−28
31	M	22	F.A.	5,730	45.5	4	3,710	27.7	−35	−39
32	M	52	F.A., emphysema	4,580	24.3	5	3,650	18.8	−20	−23
33	M	27	M.A.	4,510	30.3	4½	2,750	21.0	−39	−31
34	F	35	F.A.	3,590	24.3	4	2,850	18.3	−21	−25
Average of first postoperative test		39		4,155	21.6	4	3,080	15.0	−26	−30

* V_L is the volume of the lung in cubic centimeters of dry gas at 0° and 760 mm. at the time of breath-holding.

† D_L is the diffusing capacity of the lung for carbon monoxide, and is reported in cubic centimeters of carbon monoxide diffusing into the blood per minute per mm. Hg difference between alveolar carbon monoxide tension and blood carbon monoxide tension.

‡ Min.—minimal tuberculosis.

§ F.A.—far advanced tuberculosis.

‖ M.A.—moderately advanced tuberculosis.

temperature and pressure, dry) of the wax-pack thoracoplasty group before surgery was 4,210 cc., and that of the lobectomy group was 4,155 cc. The mean preoperative D_L of the former was 16.8, and that of the latter group was 21.6. The mean postoperative D_L of the former was 11.7, and was 15.0 for the latter. Thus it is apparent that, while the resection patients had preoperative lung volumes similar to the patients who underwent thoracoplasty, they had slightly better diffusing capacities both pre- and postoperatively; this is probably a result of their younger age and better clinical condition.

All but 3 patients had uncomplicated postoperative courses. After right upper lobectomies, 2 patients developed middle lobe atelectasis and one patient developed severe bronchospasm. However, these complaints were partially relieved before the first postoperative test, and the postoperative diffusion losses of these 3 patients paralleled the findings of the lobectomy group as a whole.

TABLE 4

PREOPERATIVE AND POSTOPERATIVE DATA ON PATIENTS UNDERGOING PNEUMONECTOMY

Case Number	Sex	Age	Diagnosis	Preoperative Data		Postoperative Data				
				V_L*	D_L†	Weeks after Surgery	V_L	D_L	Per Cent of Change	
									V_L	D_L
35	M	49	Neoplasm	4,480	16.6	3	2,255	8.4	−50	−49
36	M	67	Neoplasm	5,990	18.7	5	2,945	12.2	−51	−35
37	M	52	Neoplasm	4,830	23.9	2	2,825	13.6	−42	−43
38	M	59	Neoplasm	6,190	33.6	2½	3,285	19.5	−47	−42
39	M	42	Neoplasm	4,560	34.3	5½	3,320	21.7	−27	−37
Average of first post-operative test		54		5,210	25.4	3½	2,926	15.1	−44	−41

* V_L is the volume of the lung in cubic centimeters of dry gas at 0° and 760 mm. at the time of breath-holding.

† D_L is the diffusing capacity of the lung for carbon monoxide, and is reported in cubic centimeters of carbon monoxide diffusing into the blood per minute per mm. Hg difference between alveolar carbon monoxide tension and blood carbon monoxide tension.

DISCUSSION

The results obtained indicate a significant acute decrease in pulmonary diffusing capacity following thoracic surgical procedures. The changes following pneumonectomy are similar to those described previously for the late post-pneumonectomy period (2–4), and might be explained by the loss of functioning pulmonary parenchyma, in that the pulmonary diffusing capacity (D_L) and the breath-holding lung volume (V_L) fall to approximately the same degree. That the remaining lung fails to increase its apparent diffusing capacity when the entire cardiac output has been diverted through it, doubling its blood flow, is compatible with the concept that D_L is not related directly to pulmonary blood flow, but is apparently dependent upon intravascular pressures and the size of the pulmonary vascular bed (7–9).

It is more difficult to explain the considerable fall in D_L which follows thoracoplasty, lobectomy, or wedge resection, for in these instances the change in D_L is out of proportion to the loss of lung volume or reduction of parenchymal tissue. In this regard, it may be noted that the diffusion loss/volume loss ratio is greater in the wedge resection and plombage patients than in the lobectomy patients. The excessive diffusion loss seems to correlate with the amount of trauma to the remaining functioning lung on the operated side. Thus, in pneumonectomy no traumatized lung remains on the operated side, and in lobectomy the division of lobes with their separate blood supplies allows resection of one lobe without damage to the blood supply of the rest of that lung. Wedge resection and wax-pack thoracoplasty, however, do traumatize lung and blood supply to immediately adjacent functioning areas. As some of the thoracoplasty patients later have resections, perhaps this can be shown more clearly by comparing results from different surgical procedures in the same patient rather than by comparing two different groups of subjects. At the time of this writing, only one patient of the thoracoplasty group has had a later resection. *Patient No. 5* had the upper and middle lobes of his right lung (including the wax pack) removed. When tested six weeks after this second surgical procedure, the D_L had decreased 44 per cent, and the volume 27 per cent, from the prelobectomy test. This compares with the 40 per cent D_L and 18 per cent V_L decreases he had shown following thoracoplasty. Although this patient showed a particularly great loss of diffusion following both procedures, the results do parallel the findings of the total group of patients. The thoracoplasty and lobectomy groups both showed the same decrease in D_L following surgery, but the thoracoplasty group had less volume loss than the lobectomy patients. *Patient No. 5* showed this same trend of similar D_L loss following both procedures,

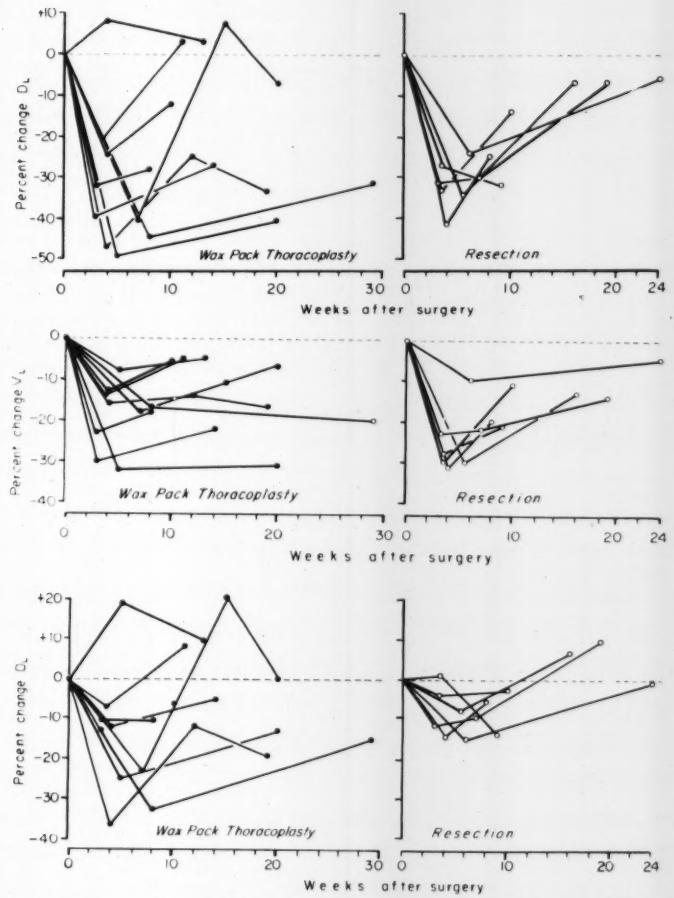

FIG. 2A (Top). Diffusion change versus postoperative time in 15 patients with repeated postoperative tests.

FIG. 2B (Center). Volume change versus postoperative time in 15 patients with repeated postoperative tests.

FIG. 2C (Bottom). Percentage of change in the pulmonary diffusing capacity (D_L) corrected for the breath-holding lung volume (V_L) change versus postoperative time in 15 patients with repeated postoperative tests. The postoperative D_L is corrected to the preoperative volume by the formula: postoperative $D_L \times$ original V_L/postoperative V_L. The percentage of difference between the corrected D_L and the preoperative D_L is shown.

but less volume loss following the thoracoplasty than following lobectomy.

The present results, which show a decreased D_L after thoracic surgery, are compatible with the findings of Schramel and associates (10) that the diffusing capacity in dogs decreases following thoracotomy and closure. They also found a decreased D_L in patients the day following open heart surgery, with a subsequent return toward normal in the following weeks. The initial decrease in many of their patients was 50 per cent or greater, but after four weeks it had returned to levels comparable with the loss of D_L seen in the present study. Thus it is possible that, in the present study, the D_L loss was even greater in the patients

TABLE 5

POSTSURGICAL V_L* AND D_L† CHANGES IN 15 PATIENTS STUDIED IN THE EARLY AND LATE POSTOPERATIVE PERIODS

	Number of Patients	Average Weeks after Surgery	Per Cent of Change	
			V_L	D_L
Plombage	9			
Early postoperative		4½	−19	−34
Late postoperative		15	−14	−17
Resection	6			
Early postoperative		4	−25	−32
Late postoperative		13	−15	−17

* V_L—breath-holding lung volume.
† D_L—pulmonary diffusing capacity.

immediately postoperatively, and had already started returning toward normal when tested.

In order to explain how this excessive loss of D_L is caused, one must hypothesize either an alteration in the alveolar-capillary membrane or a change in the distribution of the pulmonary blood supply during the early postoperative period. Previous studies have shown that a physiologic decrease in lung volume is associated with relatively little change in D_L when normal subjects perform breath-holding diffusion tests at decreasing lung volumes (11–13). Therefore, a simple inability to inspire deeply postoperatively cannot explain the obtained data. It would be possible to explain the data by a shift in vascularity from the operated to the intact lung, creating a blood supply pattern approaching that of the postpneumonectomy state. Such a mechanism might result from a temporary physiologic restriction in the pulmonary vascular bed out of proportion to the quantity of lung removed. With the passage of time, the hemodynamics of the two lungs might equalize, accounting for the subsequent increase in D_L the final loss of D_L approaching the loss of lung volume.

An acute change in the alveolar capillary membrane could similarly explain the D_L changes following surgery. One might visualize a transient interstitial pulmonary edema in the early postoperative period which resolves over a period of several weeks. Additional bronchospirometric studies of early postoperative patients would help distinguish changes in the blood-gas barrier from abnormalities in the distribution of pulmonary blood flow. To the writers' knowledge, reported postoperative bronchospirometric studies have been limited to the late postoperative period.

A third possible explanation could be a lowered hemoglobin postoperatively. A check of this revealed an average decrease in hemoglobin of 0.9 gm. following surgery. The correlation between loss of hemoglobin and excess loss of D_L is only slight ($r = 0.244$), and not of significance. Therefore, while the lowered hemoglobin cannot be ruled out as a factor influencing the reduced diffusion, it is very unlikely to be of major importance.

Regardless of the mechanisms involved, the observed fall in D_L in the early postoperative period is indicative of the profound alteration in pulmonary physiology which accompanies thoracic surgery, even wax-pack plombage. It is clear that the immediate effects of such surgery are out of proportion to the actual quantity of lung removed or collapsed. This may explain the occasional cardiorespiratory insufficiency state which develops during the early postoperative period when relatively "minor" thoracic surgical procedures are carried out in patients with pre-existing pulmonary dysfunction.

SUMMARY

The breath-holding diffusing capacities of 39 patients were obtained before and within two months after various types of thoracic surgery. Both the pulmonary diffusing capacity (D_L) and the breath-holding lung volume (V_L) decreased following lung surgery. Five patients who underwent pneumonectomy showed a comparable fall in D_L and V_L in the early postoperative period but, following thoracoplasty, wedge resection, and lobectomy there was a more marked fall in D_L than in V_L. The pulmonary diffusing capacity and the breath-holding lung volume subsequently returned toward normal, with D_L showing more improvement than V_L. Some possible mechanisms for the excessive D_L loss are discussed.

SUMARIO

Los Efectos de la Cirugía Torácica sobre la Capacidad Difusora del Pulmón

Se determinaron las capacidades difusoras de 39 enfermos mientras contenían el aliento antes

y en término de dos meses de ejecutarles varias formas de cirugía torácica. A continuación de la cirugía pulmonar, disminuyeron tanto la capacidad difusora del pulmón (D_P) como el volumen pulmonar al contener el aliento (V_P). Cinco enfermos sometidos a la neumonectomía revelaron una baja comparable de la D_P y del V_P en el período postoperatorio incipiente, pero a continuación de la toracoplastia, la resección cuneiforme y la lobectomía, fué más acentuada la baja de la D_P que la del V_P. La capacidad difusora del pulmón y el volumen pulmonar al contener el aliento volvieron después hacia lo normal, revelando la D_P más aumento que el V_P. Se discuten algunos posibles mecanismos para la excesiva pérdida de D_P.

Resume

Les effets de la chirurgie thoracique sur la capacité de diffusion pulmonaire

Les capacités pulmonaires de 39 malades, retenant leur respiration, furent obtenues avant et deux mois après différents types de chirurgie thoracique. La capacité pulmonaire de diffusion (D_L) ainsi que le volume pulmonaire quand le malade bloque sa respiration (V_L), furent diminués après chirurgie du poumon. Cinq malades qui subirent une pneumectomie montrèrent une chute comparable de D_L et V_L, au début de la période qui suivit l'opération, mais après thoracoplastie, résection partielle et lobectomie, il y eut une chute plus marquée de D_L que de V_L. La capacité pulmonaire de diffusion et le volume du poumon, à respiration bloquée, retournèrent à la normale par la suite, D_L montrant plus d'amelioration que V_L. Les auteurs discutent quelques mécanismes possibles dans la perte excessive de D_L.

Addendum

An additional 26 patients have been tested pre- and postoperatively, bringing the total number to 65. Averages for each surgical group are shown in the table below.

There is little change in the average volume or diffusion losses following surgery compared with those which are reported above. Following wedge resection, a slightly greater volume loss is noted in the present enlarged group than that reported above. Also, the enlarged group of 9 pneumonectomy patients now shows exactly the same loss of pulmonary diffusing capacity (D_L) as breath-holding lung volume (V_L) following surgery. These additional data support further the previous conclusions that, following pneumonectomy, the D_L loss corresponds to the V_L loss but, following thoracic surgical procedures of lesser extent (particularly wedge resection and plombage), the D_L loss is more marked than the loss of V_L.

Summary of the Current Preoperative and Postoperative Data on 65 Patients
Average of Each Surgical Group

Type of Surgery	Number of Patients	Preoperative Data: Age	Preoperative Data: V_L	Preoperative Data: D_L	Postoperative Data: Weeks after Surgery	Postoperative Data: V_L	Postoperative Data: D_L	Per Cent of Change: V_L	Per Cent of Change: D_L
Wax pack	12	50	4,210	16.8	5½	3,426	11.7	−19	−30
Wedge resection	9	33	3,634	17.7	5	3,207	14.4	−12	−19
Segmental resection	4	42	5,140	19.6	3½	4,240	14.1	−18	−28
Lobectomy	31	36	4,135	21.0	4	3,094	15.1	−25	−28
Pneumonectomy	9	57	4,829	23.1	4	2,592	12.5	−46	−46
Total number	65								

In order to assure the fact that reduced breath-holding volume alone cannot explain the decreased postoperative D_L, 10 patients performed the breath-holding diffusing capacity test at reduced inspiratory levels. When they voluntarily reduced their lung volumes an average of 23 per cent, a decrease of only 20 per cent in D_L occurred. However, following various thoracic surgical procedures in these same 10 patients, an average volume loss of 22 per cent was accompanied by a D_L decrease of 28 per cent. This further supports the previous conclusion that thoracic surgery produces changes in diffusion which are out of proportion to the actual quantity of lung removed or collapsed.

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THE AFTER-HISTORY OF PULMONARY TUBERCULOSIS[1]

VI. The First Fifteen Years following Diagnosis

DAVID W. ALLING[2] AND EDWARD B. BOSWORTH

(Received for publication December 21, 1959)

INTRODUCTION

In previous studies (1–3), the after-history of persons with pulmonary tuberculosis in various stages was described for the first ten years following diagnosis. Additional follow-up observations have been made on some of the patients included in these studies. The later observations afford a description of the after-history for the eleventh through the fifteenth years following diagnosis and supplement the original descriptions for the first ten years following diagnosis. The clinical material under consideration includes substantially all of the confirmed cases of active pulmonary tuberculosis that were diagnosed among residents of a semirural region in upstate New York during the years 1938 to 1948, inclusive.

Many of the persons included in the present study were patients at the Hermann M. Biggs Memorial Hospital; most of them received bed-rest treatment, and some were treated also with one form or another of pulmonary collapse. Only a few received chemotherapy, little of which was adequate by modern standards. The emphasis of the present study, however, is not on the effect or the lack of effect of any particular treatment but, rather, on the course of pulmonary tuberculosis in persons grouped according to the stage of disease at the time of diagnosis. Once the main features of the course of disease in such groups of persons are established, then the implications of these features can meaningfully be discussed.

MATERIAL

The clinical material consists of 564 persons who had active pulmonary tuberculosis at the time of diagnosis, the stage of the disease being minimal in 130, moderately advanced in 211, and far advanced in 223. The 211 persons with moderately advanced tuberculosis and the 223 with far advanced tuberculosis have been described previously (2, 3). The 130 persons with minimal disease represent a net change of 4 from the 134 previously studied (1); 12 persons who were diagnosed in 1937 have been eliminated from the present study, and 8 persons who were diagnosed in 1948 have been added. This change was made in order that the time period for admission to the study be uniform, i.e., 1938 to 1948, for all three stages of disease. The distribution of age and sex of the group of patients eliminated from the study was similar to that of the group of patients added, and no appreciable difference was noted in the follow-up experience of the two groups of patients.

The decision to limit consideration in the present paper to those who had active tuberculosis at the time of diagnosis resulted from a review of the 206 deaths from tuberculosis observed among the 941 persons included in the earlier studies (1–3). Of these deaths, only 9 occurred among the 373 persons who had arrested disease at the time of diagnosis, whereas 197 occurred among the 568 persons who had active disease at the outset. The small contribution to the tuberculosis mortality of disease that was arrested at the time of diagnosis hardly justified further efforts to obtain follow-up data on patients with this type of disease. In addition, it is not unlikely that, among persons found to have arrested tuberculosis, the variation in the duration of the arrested state prior to diagnosis exceeds somewhat the variation in the duration of active tuberculosis prior to diagnosis among persons found to have active disease. Granting this, it then follows that an initial classification of active tuberculosis is likely to represent an event more sharply defined in time than an initial classification of arrested tuberculosis. Accordingly, the latter event is of somewhat less interest.

Of the 564 persons included in the present study, 44 were not observed during their entire period of study, which began on the date of diagnosis and ended at the anniversary of diagnosis that fell in the calendar year 1956. Seventeen of these 44 persons had minimal pulmonary tuberculosis at the time of diagnosis, 18 had moderately advanced disease, and 9 had far advanced disease. Of the 44 persons, 35 are known to be alive, well, and working regularly; 3 have died of causes believed to be other than tuberculosis; while the remaining 6 have been lost sight of completely. None of the 35 persons who are apparently well and who deny symptoms of pulmonary disease will consent to have chest roentgenograms or to submit sputum for bacteriologic study.

[1] From Cornell University, Ithaca, New York.
[2] Public Health Research Fellow of the National Institutes of Health.

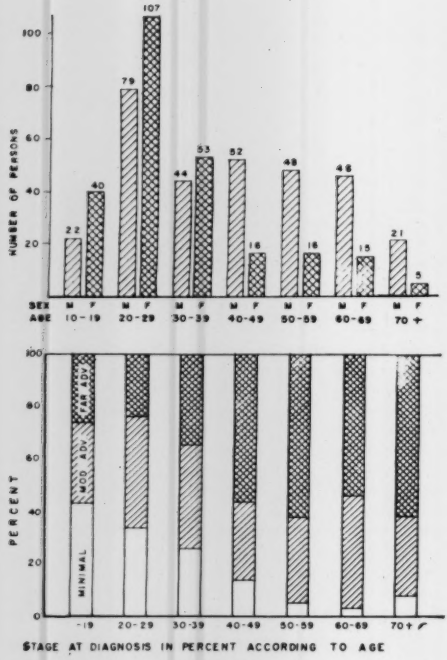


FIG. 1. (Top). Active pulmonary tuberculosis: sex and age at diagnosis.

FIG. 2. (Bottom). Stage at diagnosis (in per cent) according to age.

The distribution by sex and by age at the time of diagnosis of the 564 persons under study is shown in figure 1. The age distribution of the males is much less peaked than that of the females; however, the twenty- to twenty-nine-year age group is the largest frequency class for both sexes. Among those persons less than forty years of age there is a slight majority of females, whereas among persons older than forty years there is a marked preponderance of males. The distribution by age and sex of the persons with moderately advanced tuberculosis and of those with far advanced tuberculosis has been given previously (2, 3); the distribution by age and sex of the persons with minimal tuberculosis can be found by subtraction.

In figure 2 the percentage distribution of the three stages of tuberculosis is shown according to age at the time of diagnosis. It is seen that in the lowest age class minimal tuberculosis preponderates, whereas in the age classes from forty upward far advanced tuberculosis is more common than the other two stages combined. The latter finding is of particular importance if an observation made earlier (2, 3) is recalled, i.e., not only is the case fatality higher in tuberculosis that is far advanced at the time of diagnosis than in tuberculosis that is minimal or moderately advanced at diagnosis, but within each stage the case fatality is higher in older than in younger persons. If the clinical course of the older persons included in the present study is indicative of the experience of older persons who have recently acquired pulmonary tuberculosis, then the delay of tuberculous infection to a higher age level is not an unmixed blessing, despite the fact that such a postponement—considered as a population phenomenon—is not uncommonly associated with a lowering of the level of tuberculous contagion.

The annual incidence of new cases of active tuberculosis according to age is summarized in table 1. During the eleven-year period there was a noticeable decrease in the incidence of new cases among younger persons, a somewhat less marked decrease among middle-aged persons, and no decrease at all among older persons. The decrease in incidence observed among the group of younger persons and the group of middle-aged persons may be explained, at least in part, by a fall in the level of tuberculous contagion (*see* discussion in (3)). Now, if the fall in the level of contagion were similar for all ages, then, during the eleven-year period, the incidence of tuberculosis would be expected to decline in the older age group. (From 1940 to 1950 the total population of the eight counties included in the present study rose 3.5 per cent while, during the same period, the population fifty years and older rose 7.3 per cent (4); hence, the size of the population exposed to tuberculous contagion was essentially constant, and the annual number of diagnoses may be regarded as directly related to the level of contagion.) Thus, the question arises whether older persons are subject to the same risk of acquiring progressive tuberculosis as are younger and middle-aged persons. The question is not satisfactorily answered by assuming that the older persons acquired their infections sometime in the remote past, since the level of contagion in upstate New York—as evidenced by the tuberculosis death rate—has been falling steadily except for brief periods over the past fifty years (5). The writers suspect that, during the period of time just preceding and included by the present study in the geographic region concerned, the level of tuberculous contagion did not decrease for older persons to the same extent as for younger and middle-aged persons. It is noted that this concept is not inconsistent with a phenomenon observed in each of three earlier studies (1–3), namely, a rise over the eleven-year study period in the median age at diagnosis.

METHOD

The method of classification of the follow-up data and the statistical procedure whereby the set of classifications are summarized have been described elsewhere (6). The present writers were unaware when they derived the statistical method (the "anniversary" method) used throughout the

TABLE 1

ANNUAL INCIDENCE OF ACTIVE TUBERCULOSIS (ALL STAGES) BY AGE AT DIAGNOSIS

Age at Diagnosis	Year of Diagnosis											Total
	1938	1939	1940	1941	1942	1943	1944	1945	1946	1947	1948	
14–29	33	27	33	29	20	17	22	13	21	22	11	248
30–49	23	15	18	18	23	15	14	6	10	14	9	165
50+	11	11	14	11	12	19	17	20	10	16	10	151

Slope of best-fitting straight line is different from zero ($P > 0.99$) in the age groups 14–29 and 30–49, and is not different from zero in the 50+ age group.

series of papers, "The After-History of Pulmonary Tuberculosis," that Iskrant and his associates had previously described a similar statistical procedure (7). It should be noted that the procedure used by Iskrant and the present writers is essentially an actuarial method (*see* Berkson and Gage (8) or Kaplan and Meier (9), with the additional feature that the conditional probability is estimated that a certain classification of disease is present in a person on a particular date given that he has survived to the date in question. Previously, Stephens (10) had recognized the importance of this type of conditional probability and used estimates of it in connection with the *ad hoc* (or reduced sample) method.

In tables 2 to 4 and figures 3 to 5 are shown the percentages of persons in the various disease classifications (arrested; active, never arrested; active by relapse; died of tuberculosis; died of other cause) estimated to occur at each anniversary of diagnosis. It is assumed that the experience of those persons who, for one reason or another, pass from observation will parallel the experience of those persons who remain under observation. The data are presented separately for the groups of persons having minimal, moderately advanced, and far advanced pulmonary tuberculosis at the time of diagnosis. It should be noted that the stage of disease at subsequent anniversaries was not necessarily the same as that observed at the time of diagnosis.

RESULTS

Minimal Tuberculosis

It may be seen in table 2 and figure 3 that, from the eleventh through the fourteenth anniversaries, there are only small increases in the percentage of persons who have arrested tuberculosis and in the percentage who have died of tuberculosis, and that these increments are at the expense of the percentage of persons with active disease. At the fifteenth anniversary there is a slight decrease in the proportion of persons with arrested disease; this results from a relapse of disease in one patient classified as having arrested disease at the fourteenth anniversary and a nontuberculous death in another. Whereas at the tenth anniversary, about one person in twelve is estimated to have active disease; at the fifteenth anniversary, about one person in twenty is estimated to have active disease.

The follow-up data collected for the purpose of the present report supplied considerable additional information about the first ten anniversaries of experience following diagnosis. Furthermore, the writers succeeded in filling in a number of gaps in the information collected for the earlier study of minimal tuberculosis. These data made it possible to assign a definite clinical status at each anniversary to the persons with disease previously classified as "presumably arrested." Almost without exception, the persons so classified turned out to have arrested disease according to the criteria adopted in the original paper of this series (6).

Accordingly, the results shown in table 2 for the first ten anniversaries may be compared with the corresponding data shown in table 5 of the earlier report on minimal tuberculosis (1), with the proviso that the entries in column 11 of table 2 be compared with the *sum* of the corresponding entries in columns 11 and 12 of table 5. There appears to be reasonably fair agreement between the sets of percentages shown in the two tables.

Moderately Advanced Tuberculosis

In table 3 and figure 4 it may be seen that, from the eleventh to the fifteenth anniversaries of diagnosis, there is a small decrease in the percentage of persons with active disease which is accompanied by a small increase in the percentage of persons dead of tuberculosis. Moreover, there is a small decrease in the percentage of persons with arrested disease and a correspond-

TABLE 2

PATIENTS WITH MINIMAL TUBERCULOSIS CLASSIFIED BY STATUS AT ANNIVERSARIES OF DIAGNOSIS

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)
Anniversary	Living at Anniversary			Since Previous Anniversary		Total	Incomplete	Factors		Expected Percentages				
	Arrested	Active, Never Arrested	Active by Relapse	Dead of Tuberculosis	Dead of Other Cause			Provisional	Correction	Arrested	Active, Never Arrested	Active by Relapse	Dead of Tuberculosis	Dead of Other Cause
0	0	130	0	0	0	130	0	1.0000	0.7692	0	100.0	0	0	0
1	33	96	0	1	0	130	0	1.0000	0.7692	25.4	73.8	0	0.8	0
													0.8	
2	73	54	0	1	0	128	1	1.0078	0.7752	56.6	41.9	0	0.8	0
													1.6	
3	91	30	3	2	0	126	1	1.0079	0.7813	71.1	23.4	2.3	1.6	0
													3.2	
4	93	19	6	3	0	121	3	1.0248	0.8007	74.5	15.2	4.8	2.4	0
													5.6	
5	87	13	14	2	0	116	2	1.0172	0.8145	70.9	10.6	11.4	1.6	0
													7.2	
6	94	9	9	1	1	114	0	1.0000	0.8145	76.6	7.3	7.3	0.8	0.8
													8.0	*0.8*
7	94	7	8	1	0	110	2	1.0182	0.8293	78.0	5.8	6.6	0.8	0
													8.8	*0.8*
8	90	5	8	1	2	106	3	1.0283	0.8528	76.8	4.3	6.8	0.9	1.7
													9.7	*2.5*
9	86	5	4	1	0	96	7	1.0729	0.9150	78.7	4.6	3.7	0.9	0
													10.6	*2.5*
10	73	4	4	1	0	82	13	1.1585	1.0600	77.4	4.2	4.2	1.0	0
													11.6	*2.5*
11	66	3	4	0	0	73	8	1.1096	1.1762	77.6	3.5	4.7	0	0
													11.6	*2.5*
12	62	2	2	0	0	66	7	1.1061	1.3009	80.7	2.6	2.6	0	0
													11.6	*2.5*
13	53	0	1	1	0	55	11	1.2000	1.5611	82.7	0	1.6	1.6	0
													13.2	*2.5*
14	49	0	1	0	0	50	4	1.0800	1.6860	82.6	0	1.7	0	0
													13.2	*2.5*
15	35	0	2	0	1	38	12	1.3158	2.2185	77.6	0	4.4	0	2.2
													13.2	*4.7*

(*1*) Number of years after diagnosis.

(*2*) Number of living persons with arrested disease at anniversary of diagnosis.

(*3*) Number of living persons with active disease at anniversary who never attained the arrested status.

(*4*) Number of living persons at anniversary with active disease because of relapse.

(*5*) Number who died of tuberculosis since previous anniversary.

(*6*) Number who died of other causes since previous anniversary.

(*7*) Total number at anniversary, sum of columns (*2*) to (*6*), inclusive.

(*8*) Number of "incomplete" and lost persons at anniversary.

(*9*) Provisional factor for anniversary = (column (*7*) + column (*8*))/column (*7*).

(*10*) Correction factor for anniversary = column (*9*) × correction factor for previous anniversary. The correction factor for anniversary 0 is 100/130 = 0.7692.

(*11*) Expected percentage of persons with arrested disease at anniversary: column (*2*) × column (*10*).

(*12*) Expected percentage of persons with active disease at anniversary who never attained the arrested status: column (*3*) × column (*10*).

(*13*) Expected percentage of persons at anniversary with active disease because of relapse: column (*4*) × column (*10*).

(*14*) Expected percentage of persons who died of tuberculosis since previous anniversary: column (*5*) × column (*10*). Cumulative percentage in italics.

(*15*) Expected percentage of persons who died of other causes since previous anniversary: column (*6*) × column (*10*). Cumulative percentage in italics.

TABLE 3
PATIENTS WITH MODERATELY ADVANCED TUBERCULOSIS CLASSIFIED BY STATUS AT ANNIVERSARIES OF DIAGNOSIS

(1)*	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)
Anniversary	Living at Anniversary			Since Previous Anniversary		Total	Incomplete	Factors		Expected Percentages				
	Arrested	Active, Never Arrested	Active by Relapse	Dead of Tuberculosis	Dead of Other Cause			Provisional	Correction	Arrested	Active, Never Arrested	Active by Relapse	Dead of Tuberculosis	Dead of Other Cause
0	0	211	0	0	0	211	0	1.0000	0.4739	0	100.0	0	0	0
1	7	193	0	9	2	211	0	1.0000	0.4739	3.3	91.5	0	4.3	0.9
													4.3	*0.9*
2	83	104	2	9	1	199	1	1.0050	0.4763	39.5	49.5	1.0	4.3	0.5
													8.6	*1.4*
3	100	75	5	4	2	186	3	1.0161	0.4839	48.4	36.3	2.4	1.9	1.0
													10.5	*2.4*
4	116	49	9	3	0	177	3	1.0169	0.4921	57.1	24.1	4.4	1.5	0
													12.0	*2.4*
5	117	36	12	6	3	174	0	1.0000	0.4921	57.6	17.7	5.9	3.0	1.5
													15.0	*3.9*
6	123	26	9	5	2	165	0	1.0000	0.4921	60.5	12.8	4.4	2.5	1.0
													17.5	*4.9*
7	124	16	11	1	6	158	0	1.0000	0.4921	61.0	7.9	5.4	0.5	2.9
													18.0	*7.8*
8	124	15	11	1	0	151	0	1.0000	0.4921	61.0	7.4	5.4	0.5	0
													18.5	*7.8*
9	115	10	13	3	1	142	8	1.0563	0.5198	59.8	5.2	6.8	1.5	0.5
													20.0	*8.3*
10	102	9	9	1	2	123	15	1.1220	0.5832	59.5	5.2	5.2	0.5	1.3
													20.5	*9.6*
11	90	7	7	0	0	104	16	1.1538	0.6729	60.6	4.7	4.7	0	0
													20.5	*9.6*
12	83	6	4	1	2	96	8	1.0833	0.7290	60.5	4.4	2.9	0.7	1.4
													21.2	*11.0*
13	70	4	2	1	0	77	16	1.2078	0.8805	61.6	3.5	1.8	0.9	0
													22.1	*11.0*
14	57	3	4	1	2	67	9	1.1343	0.9987	56.9	3.0	4.0	1.0	2.0
													23.1	*13.0*
15	43	2	2	0	3	50	14	1.2800	1.2784	55.0	2.6	2.6	0	3.8
													23.1	*16.8*

* See legends in table 2.

ing increase in the percentage of persons dead of causes other than tuberculosis.

Comparison of the percentages shown in the first ten entries of columns 11–17 of table 3 with the corresponding entries of table 3 of the earlier report on moderately advanced tuberculosis (2) shows quite a satisfactory agreement. Since the material in table 3 of the present study represents a combination of data included in the earlier table with data collected after 1951, it is apparent that the additional data are fairly consistent with the earlier clinical information.

Far Advanced Tuberculosis

In table 4 and figure 5 it may be seen that, among the persons who had far advanced active tuberculosis at the time of diagnosis, there is little if any change from the eleventh through the fifteenth anniversaries in the proportion with arrested disease. There is, however, a slight increase in the proportion of persons dead of tuberculosis and a corresponding decrease in the proportion of persons with active disease.

Comparison of the first ten entries of columns 11–17 of table 4 with the corresponding entries of table 4 of the earlier report on far advanced tuberculosis (3) shows a satisfactory agreement.

Relapse Rates and Remission Rates

The form in which the follow-up experience is presented in tables 2 to 4 and figures 3 to 5 is quite straightforward and may well be the

TABLE 4

PATIENTS WITH FAR ADVANCED TUBERCULOSIS CLASSIFIED BY STATUS AT ANNIVERSARIES OF DIAGNOSIS

(1)*	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)
Anniversary	Living at Anniversary			Since Previous Anniversary		Total	Incomplete	Factors		Expected Percentages				
	Arrested	Active, Never Arrested	Active by Relapse	Dead of Tuberculosis	Dead of Other Cause			Provisional	Correction	Arrested	Active, Never Arrested	Active by Relapse	Dead of Tuberculosis	Dead of Other Cause
0	0	223	0	0	0	223	0	1.0000	0.4484	0	100.0	0	0	0
1	1	134	0	88	0	223	0	1.0000	0.4484	0.4	60.1	0	39.5	0
													39.5	
2	27	83	0	25	0	135	0	1.0000	0.4484	12.1	37.2	0	11.2	0
													50.7	
3	56	40	2	12	0	110	0	1.0000	0.4484	25.1	18.0	0.9	5.4	0
													56.1	
4	54	28	6	5	3	96	2	1.0208	0.4577	24.7	12.8	2.7	2.3	1.4
													58.4	*1.4*
5	51	23	8	4	2	88	0	1.0000	0.4577	23.3	10.5	3..	1.8	0.9
													60.2	*2.3*
6	49	20	9	3	0	81	1	1.0123	0.4633	22.7	9.3	4.2	1.4	0
													61.6	*2.3*
7	52	14	7	3	2	78	0	1.0000	0.4633	24.1	6.5	3.2	1.4	0.9
													63.0	*3.2*
8	53	8	6	4	1	72	1	1.0139	0.4697	24.9	3.8	2.8	1.9	0.5
													64.9	*3.7*
9	47	5	9	1	1	63	4	1.0635	0.4996	23.5	2.5	4.5	0.5	0.5
													65.4	*4.2*
10	42	5	7	2	0	56	5	1.0893	0.5442	22.8	2.7	3.8	1.1	0
													66.5	*4.2*
11	40	4	5	2	0	51	3	1.0588	0.5762	23.0	2.3	2.9	1.2	0
													67.7	*4.2*
12	34	3	3	1	1	42	7	1.1667	0.6722	22.8	2.0	2.0	0.7	0.7
													68.4	*4.9*
13	29	3	2	0	1	35	5	1.1429	0.7683	22.3	2.3	1.5	0	0.7
													68.4	*5.6*
14	23	3	1	1	0	28	6	1.2143	0.9329	21.5	2.8	0.9	0.9	0
													69.3	*5.6*
15	19	2	1	0	0	22	5	1.2273	1.1450	21.8	2.3	1.1	0	0
													69.3	*5.6*

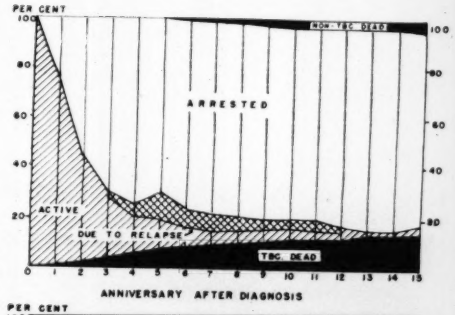
* See legends in table 2.

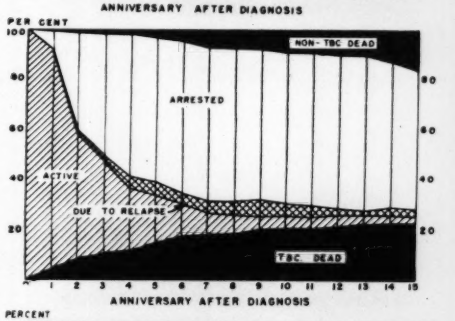
sort of information in which the physician caring for tuberculous patients is interested. However, follow-up data can be presented in another manner, namely, in terms of relapse, remission,[3] and death rates. The latter means of presentation expresses roughly the chance of a patient's moving from one status of disease to another in terms of his length of stay in the original status.

There is clearly an intimate relationship between the two methods of presentation which, after all, are but two different ways of looking at the same set of follow-up data.[4] For example, if the relapse rate decreases as the length of stay in the arrested status grows longer, and the remission rate and death rate decrease as the length of stay in the active status grows longer, then eventually the percentages of persons with active tuberculosis with arrested tuberculosis, and dead of tuberculosis will no longer change

[3] Remission is used here to denote the change in status of pulmonary tuberculosis from active to arrested. The terms "relapse rate" and "remission rate" are defined and discussed in an earlier paper (1).

[4] A more complete and somewhat more formal discussion of this subject is included in a recent paper (11) describing a stochastic model for follow-up experience in pulmonary tuberculosis.





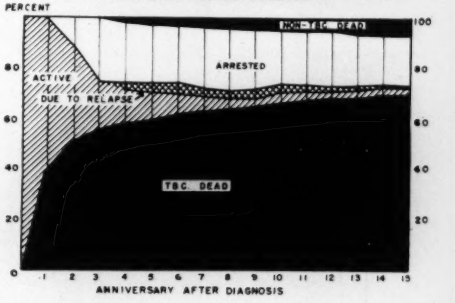


FIG. 3 (Top). Status at anniversary. Minimal tuberculosis: 130 cases.

FIG. 4 (Center). Status at anniversary. Moderately advanced tuberculosis: 211 cases.

FIG. 5 (Bottom). Status at anniversary. Far advanced tuberculosis: 223 cases.

appreciably. The converse of the foregoing statement is not necessarily true, however, since it is quite possible to have stability in the proportions of persons with active disease and of persons with arrested disease despite an appreciable (although balanced) interchange of persons between these two classifications of disease.

In tables 5 and 6, the relapse and remission rates observed among the persons included in the present study are presented. It is noted that these observed rates are estimates of the true rates and, as such, are subject to chance fluctuation. A measure of this chance variation is given by the estimated standard deviation shown in the last line of each group of entries in the two tables. The estimated standard deviations of those rates different from zero become larger in the right-hand portion of table 6 (and in one instance also in table 5) and, hence, less confidence is to be placed in the values of the rates shown there. With this proviso there appears to be an over-all decrease in the relapse and remission rates in all three groups of patients. In each case, however, the apparent decrease is gradual; hence, a fair number of relapses or remissions occur after the fifth year of arrested, active disease. Thus, the statement made in the study of far advanced tuberculosis (3)—that relapses seldom occur after five years of arrest—appears to have been overoptimistic.

Deaths Due to Causes Other than Tuberculosis

As might be expected in a sizeable group of persons observed over an extended period of time, deaths from a variety of causes were observed in the present study. Deaths believed to be due to progressive pulmonary tuberculosis or its several complications were classified as tuberculous, while the remainder were classified as nontuberculous. In table 7 the observed numbers of deaths classified as nontuberculous are compared with the numbers expected on the basis of ordinary actuarial experience; the latter were computed with the use of the 1940 United States Life Tables, table 4 (12). It is seen that, among persons who had minimal tuberculosis and among persons who had far advanced tuberculosis at the time of diagnosis, the agreement is good between the observed and expected numbers. Among persons who had moderately advanced tuberculosis at the time of diagnosis, however, an excess is seen of deaths classified as nontuberculous over the expected number of such deaths. A detailed listing of the causes of the 26 deaths observed in this group may be seen in table 8.

SUMMARY

During the years 1938–1948 in eight semirural counties in New York State, 564 persons were found to have active pulmonary tuberculosis; the disease was classified as minimal in 130, as

TABLE 5
RELAPSE RATES AMONG PATIENTS WITH TUBERCULOSIS ACTIVE AT DIAGNOSIS

Stage of Disease at Diagnosis	Number of Years of Observation													
	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Minimal														
Number of patients observed	112	103	97	89	83	77	64	60	52	48	41	31	12	7
Number of relapses*	6	5	7	4	1	2	0	2	0	0	0	0	0	1
Relapse rate (%)	5.4	4.9	7.2	4.5	1.2	2.6	0	3.3	0	0	0	0	0	14.3
Estimated standard deviation (%)	2.1	2.1	2.6	2.2	1.2	1.8	0	2.3	0	0	0	0	0	13.2
Moderately advanced														
Number of patients observed	147	136	123	116	108	103	91	76	61	57	45	35	21	3
Number of relapses*	9	11	3	5	2	2	0	0	1	2	0	0	0	0
Relapse rate (%)	6.1	8.1	2.4	4.3	1.9	1.9	0	0	1.6	3.5	0	0	0	0
Estimated standard deviation (%)	2.0	2.3	1.4	1.9	1.3	1.4	0	0	1.6	2.4	0	0	0	0
Far advanced														
Number of patients observed	71	61	52	48	44	39	35	32	30	28	26	20	9	8
Number of relapses*	6	6	3	1	1	2	1	1	0	0	0	0	0	0
Relapse rate (%)	8.5	9.8	5.8	2.1	2.3	5.1	2.9	3.1	0	0	0	0	0	0
Estimated standard deviation (%)	3.3	3.8	3.2	2.1	2.2	3.5	2.8	3.1	0	0	0	0	0	0

* Number of relapses occurring in final year of observation period. The patients experiencing a relapse had arrested disease during the portion of the observation period prior to the final year. The remaining patients had arrested disease throughout the observation period.

TABLE 6
REMISSION RATES AMONG PATIENTS WITH TUBERCULOSIS ACTIVE AT DIAGNOSIS

Stage of Disease at Diagnosis	Number of Years of Observation													
	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Minimal														
Number of patients observed	127	52	26	16	13	8	7	5	4	3	2	0	0	0
Number of remissions*	73	22	7	3	4	1	2	0	1	1	0	0	0	0
Remission rate (%)	57.5	42.3	26.9	18.7	30.8	12.5	28.6	0	25.0	33.3	0	0	0	0
Estimated standard deviation (%)	4.4	6.8	8.7	9.8	12.8	11.7	17.1	0	21.6	27.2	0	0	0	0
Moderately advanced														
Number of patients observed	189	98	71	42	32	22	16	11	9	8	6	5	3	2
Number of remissions*	85	23	22	6	6	6	1	1	0	1	0	1	0	0
Remission rate (%)	45.0	23.5	31.0	14.3	18.7	27.2	6.3	9.1	0	12.5	0	20.0	0	0
Estimated standard deviation (%)	3.6	4.3	5.5	5.4	6.9	9.5	6.1	8.7	0	11.7	0	17.9	0	0
Far advanced														
Number of patients observed	110	71	34	24	21	17	10	7	5	4	3	3	3	2
Number of remissions*	28	30	6	1	1	3	2	2	0	0	0	0	0	0
Remission rate (%)	25.4	42.3	17.7	4.2	4.8	17.6	20.0	28.6	0	0	0	0	0	0
Estimated standard deviation (%)	4.1	5.9	6.5	4.1	4.6	9.2	12.6	17.1	0	0	0	0	0	0

* Number of remissions occurring in final year of observation period. The patients experiencing a remission had active disease during the portion of the observation period prior to the final year. The remaining patients had active disease throughout the observation period.

TABLE 7

COMPARISON OF OBSERVED AND EXPECTED NUMBER OF DEATHS DUE TO CAUSES OTHER THAN TUBERCULOSIS

Stage of Disease at Diagnosis	Number of Deaths	
	Observed	Expected
Minimal	4	4.15
Moderately advanced	26	16.95
Far advanced	11	10.24

TABLE 8

NONTUBERCULOUS DEATHS BY CAUSE AMONG TUBERCULOUS PATIENTS WITH DISEASE OF MODERATELY ADVANCED EXTENT AT TIME OF DIAGNOSIS

Cause of Death	Number of Deaths	
Cardiovascular—renal disease:		16
Congestive failure:	7	
Arteriosclerotic heart disease, 5		
Syphilitic heart disease, 1		
Rheumatic heart disease, 1		
Coronary artery occlusion and/or myocardial infarction	4	
Cerebrovascular accident	1	
Pulmonary embolism (following childbirth)	1	
Renal disease:	3	
Associated with diabetes mellitus, 1		
Amyloidosis associated with rheumatoid arthritis, 1		
Sulfonamide toxicity, 1		
Neoplastic disease:		5
Prostate	1	
Breast	1	
Melanoma	1	
Stem cell leukemia	1	
Primary site undetermined	1	
Miscellaneous:		3
Perforated duodenal ulcer	1	
Accidental	1	
Suicide	1	
Possibly tuberculous:		2
These two persons died at home of "bronchopneumonia" and "heart disease" after short periods (1–2 years) of active, progressive tuberculosis. Their ages were 74 and 80 years, respectively.		

moderately advanced in 211, and as far advanced in 223. At the time of diagnosis among those persons aged thirty-nine years or less, the disease was far advanced in two-sevenths, and females outnumbered males four to three. Among those persons aged forty years and older, the disease was far advanced in three-fifths, and males outnumbered females three to one. During the eleven-year study period, the annual incidence of cases was found to decrease markedly in the age group of fourteen to twenty-nine years, to decrease moderately in the age group of thirty to forty-nine years, and not to decrease in the age group of fifty years and older.

In the analysis of the follow-up data, persons included in the study were grouped according to the stage of pulmonary tuberculosis at the time of diagnosis (minimal, moderately advanced, far advanced). There is a noticeable similarity in the follow-up experience of the three groups from the eleventh through the fifteenth anniversaries of diagnosis; in each group during this period there was a small decrease in the proportion of persons with active disease, a smaller decrease in the proportion of persons with arrested disease, and minor increases in the proportions of persons who died from tuberculosis and those who died from other causes.

Both the relapse rates and the remission rates observed among the three groups of persons tended to decrease as the length of time in the arrested or active state of tuberculosis increased. An excess of deaths from causes believed to be other than tuberculosis was observed during the period of study among persons classified at the time of diagnosis as having moderately advanced tuberculosis.

SUMARIO

La Historia Subsecuente de la Tuberculosis Pulmonar. VI. Los Primeros Quince Años Consecutivos al Diagnóstico

Durante los años 1938–1948 en ocho condados semirrurales del Estado de Nueva York, se descubrió a 564 personas que tenían tuberculosis pulmonar activa; se clasificó la enfermedad como mínima en 130, como moderadamente avanzada en 211 y como muy avanzada en 223. Al hacerse el diagnóstico, entre aquellas personas que tenían treinta y nueve años de edad o menos, la dolencia estaba muy avanzada en dos séptimas partes de ellas y las mujeres excedían a los varones en la proporción de cuatro a tres. Entre aquellas personas de cuarenta años o más, la enfermedad estaba muy avanzada en tres quintas partes y los varones excedían a las mujeres en la proporción de tres a uno. Durante el período de once años de estudio, se observó que la incidencia anual de casos disminuía notablemente en el grupo de catorce a veintinueve años de edad, disminuía moderadamente en el grupo de treinta a cuarenta

y nueve años y no disminuía en el grupo de cincuenta años o más de edad.

En el análisis de los datos subsecuentes, se agrupó a las personas conforme al período de la enfermedad en que se hallaban en la fecha del diagnóstico (mínimo, moderadamente avanzado, muy avanzado). Existe una notable semejanza en lo sucedido a los tres grupos en el período de observación partiendo del undécimo hasta el décimoquinto aniversario del diagnóstico inclusive; en cada grupo, durante ese período, hay una pequeña disminución en la proporción de personas que tienen enfermedad activa, una disminución menor en la proporción de las que tienen la enfermedad estacionada y menores aumentos en las proporciones de personas que han fallecido de la tuberculosis y de las que han fallecido de otras causas.

Los coeficientes tanto de recidivación como de remisión observados en los tres grupos de personas tienden a disminuir a medida que aumenta el largo de tiempo en la fase estacionada o activa de la tuberculosis. Durante el período de estudio, se observó un exceso de muertes por causas juzgadas como distintas de la tuberculosis entre las personas clasificadas al hacerse el diagnóstico como afectadas de tuberculosis moderadamente avanzada.

Resume

Les suites de la tuberculose pulmonaire. VI. Les quinze premières années après le diagnostic

Pendant les années de 1938 à 1948, dans huit départements semi-ruraux de l'état de New York, on trouva 564 personnes ayant une tuberculose pulmonaire active; la maladie fut enregistrée comme minimum dans 130 des cas, modérément avancée dans 211 cas et très avancée dans 223. Au moment du diagnostic, parmi les personnes agées de 39 ans ou moins, la maladie fut trés avancée dans les deux septièmes des cas, et il y eut plus de femmes que d'hommes dans la proportion de trois pour quatre. Parmi les personnes agées de plus de quarante ans et plus, la maladie fut plus avançée dans les trois cinquièmes des cas et les hommes furent plus nombreux que les femmes dans la proportion de trois pour une. Pendant la période d'étude de 11 ans, on trouva que la fréquence annuelle des cas décrut notablement dans le groupe agé de 14 à 29 ans, et ne décrut pas dans le groupe agé de 50 ans et plus.

Dans l'étude des renseignements sur les suites, les personnes étudiées furent groupées suivant le stade de tuberculose pulmonaire au moment du diagnostic (minimum, modérément avançé, trés avançé). Il y a une similarité notable dans les suites des trois groupes de 11 à 15 ans aprés le diagnostic; dans chaque groupe, pendant cette période, il y eut une légère diminution chez les personnes ayant une forme active, une diminution moins grande chez les personnes ayant une forme fixée, et des augmentations mineures chez des personnes qui moururent d'autres causes.

Les taux de rechute et de rémission observés parmi les trois groupes tendent à décroitre à mesure que les durées de la stabilisation de la maladie ou de l'activité de la maladie augmentent. Un nombre de décès surnuméraires, de causes autres que la tuberculose, fut observé pendant la période d'étude parmi les personnes classées au moment du diagnostic comme porteurs d'une forme de tuberculose modérément avançée.

Acknowledgment

The writers are indebted to the staffs of Mt. Morris Tuberculosis Hospital, Dr. Frank L. Armstrong, Director; and Onondaga Sanatorium, Dr. Bernard T. Brown, Director; and to a number of county health officers, district health officers, and public health nurses in upstate New York for their generous help in collecting follow-up information. They are also indebted to those Veterans Administration facilities, out-of-state health departments, and private physicians who made medical records and chest roentgenograms available.

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RESECTIONAL THERAPY FOR RESIDUAL NONINFECTIOUS CAVITARY TUBERCULOUS LESIONS[1, 2]

WATTS R. WEBB, JESSE L. WOFFORD, AND HANS-KARL STAUSS

(Received for publication November 13, 1959)

Introduction

The classification of residual tuberculous lesions according to the bacillary and cavitary status aids in objectively evaluating each case. While the susceptibility or resistance of the bacilli to antituberculous drugs is probably the most important factor influencing operative results, the presence or absence of a residual cavity is probably the most important factor in the final outcome of medical therapy. Bell, Decker, and Raleigh (1) found that 43 per cent of 118 patients who had residual cavities and negative bacteriologic studies had relapses during the next three years, more than half occurring during active chemotherapy. Most relapses occurred early, nearly 75 per cent within one year of the observation point and more than 90 per cent within two years of that time. Of 153 similar cases of patients treated with resection, only 9 per cent developed subsequent tuberculous manifestations. As 41 per cent of the resected cavities each showed a culture positive for tubercle bacilli, it is obvious that the bacilli in these lesions had not been sterilized. Other investigators (2–7), using continuous medical therapy with different standards of reference, have demonstrated low relapse rates. Corpe and Blalock (2), in following patients who had been continuously noninfectious for an average of sixteen months prior to onset of the study, found a relapse rate of only 8.8 per cent. Most of the relapses in Bell's series (1), however, had already occurred prior to the date of onset of this study. Similarly, in the present material the great number of patients who have had bacteriologic, clinical, or roentgenographic spread of their disease shortly after attaining the noninfectious state has been impressive. The lesions in such cases frequently become inoperable, at least temporarily, long before the patient attains the "open-negative" state studied by Corpe and others. Some of these patients become noninfectious again and are candidates for resection. Others remain infectious and may have surgical treatment under protection of additional drugs, but at a higher risk (8).

To evaluate the surgical results in patients in the present study who have residual cavities, but otherwise have reached the goal of roentgenographic stability and noninfectious status, all such resections performed during the four and one-half year period from 1955 through June, 1959, have been analyzed.

Composition of Series

Two hundred operations were performed on 188 patients. Only those patients are included who had pathologic demonstration of air-containing cavities, regardless of the preoperative roentgenographic interpretation, as various solid caseous lesions were found to have the roentgenographic appearance of being air-containing (9). Bronchiectatic lesions, blebs, bullae, or other cysts distinguishable as such, are not included. All bacteriologic studies of sputum and gastric washings for at least two months immediately prior to operation were negative, although many patients had noninfectious sputum for several months prior to operation. Thus, not all met the criteria of the "open-negative" syndrome, as defined at present by the Committee on Therapy of the American Trudeau Society (10), as an open air-containing lesion with tubercle bacilli absent from the sputum for at least six months. Operation was usually offered as soon as the "target point" of a fairly stable roentgenogram and negative bacteriology was achieved, and no operation was deliberately delayed for further study.

Of these 188 patients (table 1), 130 were receiving initial antimicrobial therapy, and 58 were receiving re-treatment. The much higher proportion of Negroes in the re-treatment group is a reflection of the difficulty in obtaining their cooperation in long-term medical care, as drug therapy was usually instituted on the "home care" program some time prior to admission to the sanatorium. Other than this, the marked differences between the initially treated and re-treated groups noted by Steininger and Howard (12) were not observed. The re-treated patients

[1] From the Departments of Surgery, Mississippi State Sanatorium and University of Mississippi Medical Center, Jackson, Mississippi.

[2] Presented at the annual meeting of the Southern Trudeau Society, Biloxi, Mississippi, October 1, 1959.

TABLE 1

RACE AND SEX OF 188 SURGICAL PATIENTS WHO RECEIVED INITIAL TREATMENT OR RE-TREATMENT WITH ANTIMICROBIAL DRUGS

Sex	Race	Initial Treatment	Re-treatment
Male	White	46	10
Female	White	28	7
Male	Negro	28	20
Female	Negro	28	21
Total		130	58

often had to be classified as such on their initial admission and were largely a product of the early developmental concepts of chemotherapy and of "home treatment," rather than recalcitrant patients.

It would have been ideal to have evaluated the initial response to institutional therapy and to have completed surgery, when indicated, before the end of the first year. In this series, such was possible in only 36 (19 per cent) of the 188 patients.

The extent of the resections is shown in table 2, illustrating the large number of pneumonectomies and other major resections done, particularly in the re-treatment group.

Seven patients had bilateral resections, while 5 of the patients were reoperated upon on the same side (table 3). One of the latter proved to have a closed lesion, negative for tubercle bacilli, while the other 4 (all in the re-treatment series) had developed further cavitary lesions, with demonstrable bacilli of proved resistance to streptomycin, isoniazid, and PAS. It is probable that in these cavities the bacilli were already resistant at the time of the first surgical procedure.

OBSERVATIONS

There have been seven deaths with only one attributable to active tuberculosis (table 4). The early deaths in the initially treated group were due to retained secretions on the first postoperative day, to massive hemorrhage from a duodenal ulcer at one month, and to proved pulmonary emboli at five months postoperatively. The late death of a 61-year-old male occurred from acute myocardial infarction four days after discharge and six months after surgery. Two early deaths in the re-treatment group were due to pulmonary edema at thirty-six hours and to pneumonia four days postoperatively. The last death of a re-treated patient occurred fourteen months postoperatively from massive pulmonary hemorrhage following definite reactivation of tuberculosis.

The complications are presented in table 5. Ten of the patients had sufficient difficulty with retained secretions to require repeated bronchoscopies or tracheostomy, the latter being performed in 9 patients because of secretions or respiratory insufficiency. One bronchopleural fistula occurred after a right upper lobectomy when the extrapleural Ivalon plombage became infected. This closed immediately with removal of the sponge and conversion thoracoplasty. The other two fistulas closed with thoracoplasties. Nine patients had difficulty with pulmonary re-expansion, but responded within two weeks to aspirations or reintubation. Four had hemothoraces of sufficient magnitude to necessitate enzymatic debridement.

Fourteen patients have had post-surgical reactivations of their disease, equally divided between the early and late periods. In only 2 is the disease active at this time, the others having responded to further drug or surgical therapy. Of the 20 patients who did not receive isoniazid preoperatively, the disease of 6 has relapsed, reflecting the superior effectiveness of this drug.

Four patients have been lost to follow-up observation, although all were doing well when last seen (table 6). Seven of the remaining 184 patients are dead, 2 are presently active, and 175 (93 per cent) are progressing satisfactorily. One

TABLE 2

EXTENT OF OPERATIONS PERFORMED

Operation	Initial Treatment	Re-treatment
Pneumonectomy	8	18 (2)*
Lobe resected	61 (2)	30 (2)
More than one lobe resected	17 (1)	6
Segmental resection	49 (2)	9 (2)
Wedge resection	1 (1)	1
Total	136	64

* Numbers in parentheses refer to patients in the preceding group who had second procedures.

TABLE 3

REOPERATIONS PERFORMED

	Initial Treatment	Re-treatment
Contralateral	5	2
Ipsilateral	1	4*

* All cavitary lesions with bacilli resistant to streptomycin, isoniazid, and PAS at reoperation.

TABLE 4
DEATHS

	Nontuberculous		Tuberculous	
	Number	Per Cent	Number	Per Cent
Initial treatment				
Early	3	2.3	0	0
Late	1	0.8	0	0
Re-treatment				
Early	2	3.4	0	0
Late	0	0	1	1.7

TABLE 5
COMPLICATIONS OF OPERATIONS

Complication	Initial Treatment	Re-treatment
Retained secretions	6	4
Bronchopleural fistula	1	2
Empyema	1	1
Delayed expansion	6	3
Hemothorax	4	1
Tracheostomy	5	4
Cardiac arrest	0	1

TABLE 6
FOLLOW-UP STUDY OF SURGICAL PATIENTS

	Initial Treatment	Per Cent	Re-treatment	Per Cent
Dead	4	3.1	3	5.1
Lost	2	1.5	2	3.4
Reactivated	10	7.7	4	6.8
Now active	1	0.7	1	1.7
Living and well	123	94.6	52	89.6
Always well	114	87.6	49	84.7
Total living and well	175			93.1

hundred and sixty-three of the patients (86.9 per cent) have been well consistently since surgery, 12 more being converted by further antimicrobial or surgical therapy.

Acid-fast bacilli have been demonstrable by microscopy, culture, or both, in 42.5 per cent of the resected specimens which have been studied, with no difference between the initially treated and re-treatment cases. The postoperative course and long-term results likewise did not correlate with the demonstration of bacilli in the specimens except for the 4 patients with drug-resistant bacilli mentioned above who required later resections on the same side. These cases again illustrate that it is possible to suppress bacillary discharge, even of resistant organisms which have full power for subsequent reactivation of disease. Unfortunately, catalase studies are not available on this group to help determine their possible significance.

No correlation was found between the ultimate results and the size of the cavity at the time of admission or at the time of operation. Similarly, there was no significant difference in the cavitary size in the initially treated and the re-treatment groups. A higher number of the re-treatment group had associated bronchiectasis.

No correlation was found between the ultimate results and the duration of disease prior to the institution of effective antimicrobial therapy.

As antimicrobial therapy was discontinued one year postoperatively in nearly all instances, these cases afford an evaluation of the long-term results of surgery without indefinitely prolonged drug treatment in a socio-economic group that usually returned to active physical labor.

DISCUSSION

The description by Auerbach and his associates (11) of the phenomenon of "open healing" of a cavity after prolonged chemotherapy (particularly with isoniazid) with complete obliteration of all stigmata of tuberculosis, introduced a new concept into antituberculous therapy. Prior to that time all cavities had been accepted as residual foci of active infection. DeFriez, Patton, Welch, and Badger (12), in following patients with and without cavities treated without antimicrobials or surgery in the 1930's, found a cumulative relapse rate of 32 per cent at five years and more than 50 per cent at twenty years. Numerous reports, including those of Corpe, Dooneief, Cohen, Worobec, Breuer, and their associates (2–5, 7), testify to the low incidence of reactivations in patients with pulmonary cavities and negative bacteriology if a follow-up study is started six to sixteen months after the development of the noninfectious state and if effective chemotherapy including isoniazid can be continued indefinitely. It is not believed that this represents uniform achievement of "open healing," nor is such claimed, but these patients do remain well clinically and bacteriologically.

While this is a real phenomenon—and certainly of vital importance from an epidemiologic standpoint—it gives a distorted picture as it

excludes the many relapses that occur during the early period after initial reversal of infectiousness. These patients are all too frequently concealed from view and completely obscured by the late start of the long-time follow-up study of the "open-negative" group under active medical therapy. Most of these results are dependent upon the long and continuous use of antituberculous therapy, which will not be feasible in various segments of our population, even if possible. Also, while it is true that many of these "open-negative" patients can be offered surgery under the protection of additional effective drugs should a relapse occur, others in the writers' experience have been lost to surgery because of the degree of the subsequent spread of disease. In addition, both the immediate and long-term results of surgical therapy of the group positive for drug-resistant tubercle bacilli are far inferior to the results in the noninfectious group. While the demonstration of the safety of the open-negative state will be of vital importance to patients unable to have surgery for various reasons (physical or socio-economic), it does not offer the ideal with respect to therapy. This might be regarded as a method of producing "good chronics" from patients who otherwise would have died of the disease or remained active public health menaces.

Undoubtedly, with further study it will be possible to evaluate those lesions which are safest on long-term antimicrobial therapy. For example, Breuer, Abeles, Chaves, and Robins (7) found that reactivation was uncommon if the cavities were thin-walled or had a diameter greater than 5 cm. They noted a 4 per cent relapse rate in patients with thin-walled cavities, but a 19 per cent rate in patients other than those with thin-walled cavities.

The bacteriology of resected specimens will continue to be of the utmost importance. Bell, Decker, and Raleigh (1) found that 41 per cent of their resected specimens showed cultures positive for tubercle bacilli, 17 per cent of the "clean" cavities but 62 per cent of the "dirty" cavities being positive. There have been various reports that the yield has steadily increased as cultural methods have been refined. There are few study rates available of the bacteriology of lesions resected after longer periods of sputum negative for tubercle bacilli. Cohen and associates (4), on following forty-nine cases for six to eighteen months, found bacteriologic relapse in six patients. Three patients had had resections and all had tubercle bacilli in the sputum demonstrated, in one by microscopy and in two by culture. One of his patients who died of a heart attack after six years of therapy as an open-negative case had demonstrable bacilli in the residual cavitary lesion. Catalase-negative organisms, even if resistant, appear far less virulent, but the statistical and epidemiologic significance of this observation remains to be determined (14).

The present writers' observations suggest that resection can and probably should be performed for the cavitary tuberculous lesion even in the absence of demonstrable bacilli, with the expectation of satisfactory initial and late results for both the initially treated and re-treatment groups. In both categories the results appear superior to the published results of long-term nonsurgical therapy, and suggest that residual cavities, regardless of the noninfectiousness of the sputum, should be resected. Indefinitely prolonged postoperative antimicrobial therapy now seems to be the preferred treatment. As the above results were obtained with antimicrobial therapy for only six months to one year, it would seem that the patient with a cavitary residual is better protected by surgery and even by short-term treatment than by indefinitely prolonged medical therapy alone. In view of the socio-economic and practical difficulties of maintaining indefinite drug therapy, it can at best be regarded as a less satisfactory substitute for judicious, properly timed resectional therapy.

Summary

Two hundred operations were performed on 188 noninfectious tuberculous patients with cavitary residual lesions. Follow-up study from three to fifty-seven months showed about equal results in the initially treated and re-treatment groups so long as there was adequate drug control. At this time, 175 (93.1 per cent) appear to be free from disease as a result of surgery and limited postoperative antimicrobial therapy, with a total of 7 deaths and 14 reactivations.

This regimen of resecting cavitary residual lesions appears preferable to long-term antimicrobial therapy alone. It allows a high incidence of early reactivations and a late incidence equivalent to, or higher than, that reported here.

Sumario

La Resección Terapéutica para las Lesiones Tuberculosas Cavitarias No Infecciosas Residuales

Se ejecutaron 200 operaciones en 188 tuberculosos no infecciosos que tenían lesiones cavitarias residuales. El estudio subsiguiente durante tres a cincuenta y siete meses reveló resultados aproximadamente iguales en los grupos tratados inicialmente y en los re-tratados en tanto había adecuado dominio terapéutico. En la actualidad, 175 (93.1 por ciento) parecen hallarse exentos de la enfermedad a consecuencia de la cirugía y de una limitada terapéutica antimicrobiana postoperatoria, con un total de 7 defunciones y 14 reactivaciones.

Este régimen de resecar las lesiones cavitarias residuales en el "punto focal" parece preferible a la terapéutica antimicrobiana a largo plazo por sí sola, que permite una alta incidencia de reactivaciones tempranas y una incidencia tardía equivalente a la presentada aquí, sino más alta.

Resume

Traitement par résection dans les cas de cavernes tuberculeuses résiduelles, non infectées

Deux cent opérations furent effectuées chez 188 malades ayant comme lésions résiduelles des cavernes non infectées. Des études poursuivies pendant trois à cinquante sept mois montrèrent des résultats à peu près identiques chez les groupes traités initialement et ceux re-traités à condition qu'il y eut un contrôle convenable par le traitement. Au moment de l'étude, 175 (93,1%) apparurent guéris à la suite de l'opération et d'une thérapeutique antimicrobienne limitée, postopératoire avec un total de sept morts et quatorze réactivations.

Ce traitement par résection des cavernes résiduelles semble préferable à un seul long traitement antimicrobien avec lequel il y a une grande fréquence de réactivations précoces et de réactions tardives supérieures ou égales à celles rapportées dans cette étude.

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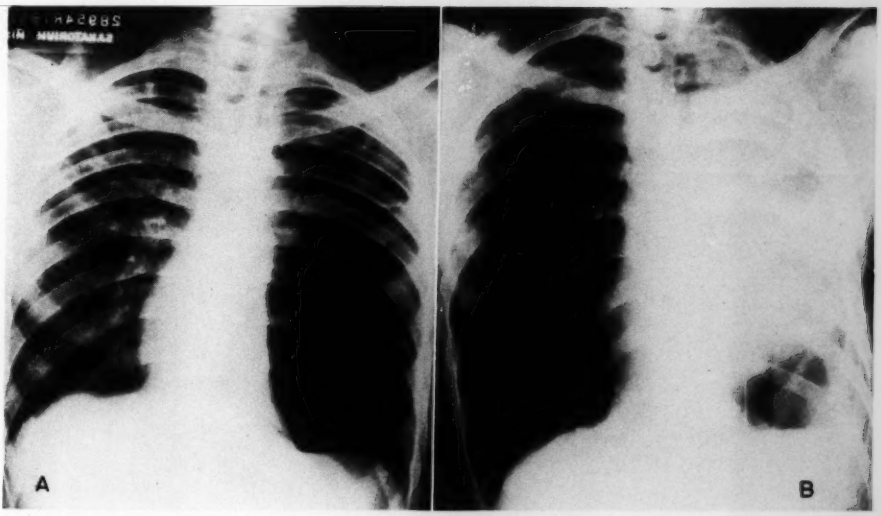

FIG. 1. Seventeen-year-old Negro male; onset of disease in March, 1953. The patient received streptomycin, isoniazid, and PAS from May, 1953, until six months after surgery. His sputum was positive for tubercle bacilli before admission, but never since then. He had a left pneumonectomy on October 1, 1956. *A*. Roentgenogram, September 28, 1954, showing "vanishing" lung on the left and granular, almost miliary, disease on the right. *B*. Roentgenogram, August 17, 1959. The patient remains asymptomatic and at full-time manual labor.

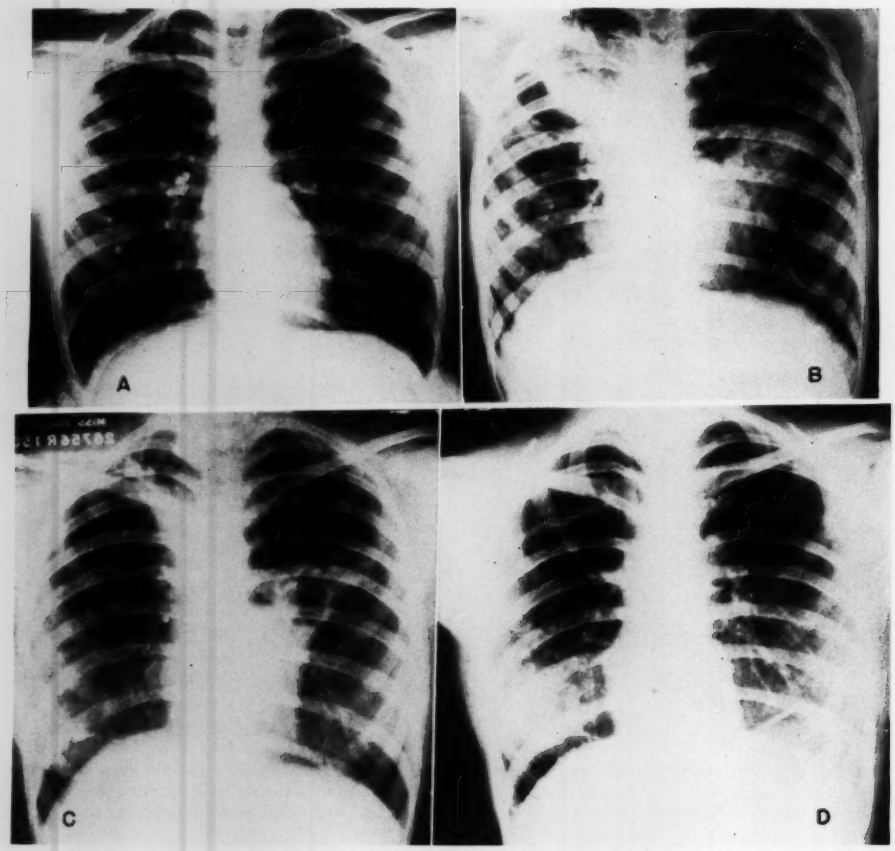

FIG. 2. Seventeen-year-old white female; onset of disease in 1949. The patient has a small lesion at the right apex. *A*. Roentgenogram, December 9, 1949. The patient received no antituberculous drugs, but pneumoperitoneum was continued for three years. *B*. Roentgenogram, February 24, 1954, showing reactivation with sputum positive for tubercle bacilli. She was readmitted to the hospital in October, 1954. Isoniazid and PAS were started and, in July, 1955, streptomycin was added. The last culture positive for tubercle bacilli was obtained in December, 1955. *C*. Roentgenogram, July 26, 1956, showing residual pneumonic and cavitary lesions bilaterally. A left upper lobectomy was performed on July 27, 1956, and a right upper lobectomy, on December 14, 1956. Drugs were continued eight months post-operatively. *D*. Roentgenogram, July 30, 1957. The patient remains asymptomatic, has two children, and does full housework.

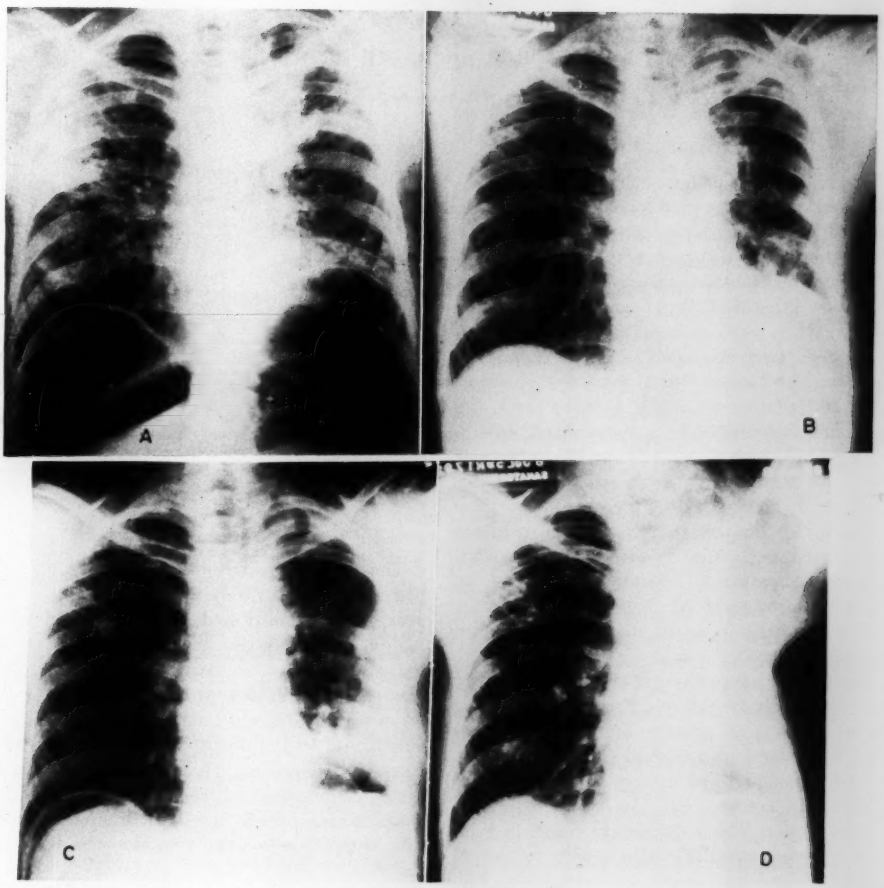

FIG. 3. Forty-eight-year-old white male epileptic. First admission to the hospital was on October 2, 1951; he remained until December 1, 1953. The patient received streptomycin–PAS and pneumoperitoneum. *A*. Roentgenogram, January 25, 1952. The patient was readmitted on October 6, 1954, and was re-treated with streptomycin–PAS; his sputum became noninfectious in August, 1956. *B*. Roentgenogram, April 6, 1957, two days prior to left upper lobectomy and wedge resection of apex of left lower lobe. *C*. Roentgenogram, April 22, 1957, showing initial favorable response, although residual fibrosis is evident. Massive spread occurred two months postoperatively. Sputum became positive for tubercle bacilli which were culturally resistant to streptomycin and PAS. Completion pneumonectomy and thoracoplasty were performed on February 2, 1959, with preoperative addition of isoniazid, cycloserine, and pyrazinamide. Cycloserine was discontinued after six weeks because of nausea and vertigo, and pyrazinamide was discontinued after five months. *D*. Roentgenogram, July 8, 1959, five months postoperatively. All bacteriologic studies are negative and the patient remains asymptomatic. The bronchiectatic lesion of the right upper lobe remains stable.

THE CALCIFIED HILAR NODE: ITS SIGNIFICANCE AND MANAGEMENT[1]

A Review

JOHN STORER AND RALPH C. SMITH

(Received for publication July 23, 1959)

Introduction

Seldom in medicine has such an easily identifiable and potentially destructive lesion as the calcified hilar lymph node been given so benign a designation. The roentgenographic detection of hilar node calcification has received little consideration other than to suggest that a healed inflammatory process has become safely incarcerated. True, the calcific focus is chemically inert and, for the most part, incapable of stimulating further tissue reaction. It is, however, the *force vitale* represented by a sharp, hard mass in contact with delicate moving intrathoracic structures which gives significance to the calcified hilar node. In the present paper it is proposed to explore the mechanism of node calcification with the development of related secondary disease and, further, to suggest an effective employment of diagnostic methods for use in the decision as to the necessity for, or type of, therapy.

Tissue necrosis is thought to be a necessary precursor to dystrophic calcification. Because of alteration in pH, oxygen tension, and other little understood phenomena, calcium salts may be laid down in these sites in a chemical composition similar to bone. The types of inflammatory lesions which ultimately result in hilar node necrosis and calcification usually have their origin in intrapulmonary disease states. Pulmonary tuberculosis, until recently, was considered the usual etiologic factor, but in recent years it has been shown that infections due to *Histoplasma capsulatum* may be a frequent cause. This may be particularly true in the Ohio Valley where histoplasmosis is endemic. Baum and his associates (1) have used special stains to demonstrate *Histoplasma capsulatum* in an expectorated broncholith which had originated as a calcified hilar node. It is their opinion that hilar node calcification, at least in the Ohio Valley, is more often related to histoplasmosis than to tuberculosis.

The calcified hilar node may be in juxtaposition to any intrathoracic organ, but those most frequently involved are the esophagus, superior vena cava, and tracheobronchial tree (2). The inflammatory process in the node antedating the calcific deposit causes the surrounding tissue to become adherent to it. Any one of several clinically dissimilar situations may thereby arise, such as superior caval obstruction, traction diverticulum of the esophagus, and erosion or compression of a bronchus. Of course, since these lesions have a common origin, they may occur concomitantly.

Middle Lobe Syndrome

The anatomic distribution of hilar and peribronchial lymph nodes is quite variable. However, the relation of nodes to the bronchi of the middle lobe and the anterior segment of the upper lobe remains fairly uniform. Compression of either of these bronchi will result in collapse of the involved lobar segment. The bronchus distal to the obstructed area may undergo classic bronchiectatic change because of the inevitable inflammatory change set up in a nondraining area. Such involvement of the middle lobe with its attendant complications has been called the middle lobe syndrome (3, 4). The symptomatology associated with this lesion consists chiefly of cough, chest pain, and recurrent febrile episodes. Wheezing may be localized over the middle lobe if the obstruction is not complete. Hemoptysis may result from the bronchiectatic change or erosion of the bronchus by a calcified node. The diagnosis may be confirmed by bronchoscopic and bronchographic examination. Extirpation of the involved lobe will in most cases result in a complete cure.

Figure 1 represents a segment of the chest film of a 45-year-old white female. She had been bothered by a cough for twenty years. The cough was intermittently productive of yellowish mucoid sputum, and on several occasions there was

[1] From the Department of Thoracic and Cardiovascular Surgery, Huron Road Hospital, Cleveland, Ohio.

FIG. 1. Segment of roentgenogram of patient with middle lobe syndrome due to obstructing calcified node.

frank hemoptysis. She had recurrent "pleurisy" almost yearly and, characteristically, spent eight to twelve weeks in bed with respiratory infections each winter. She was quite thin and had not been able to gain weight.

She was hospitalized a few weeks after having recovered partially from an acute respiratory infection characterized by cough, right chest pain, and elevation of temperature. A chest film disclosed apparent collapse of the middle lobe and scattered hilar calcifications. Bronchoscopy revealed no evidence of an endobronchial lesion; the middle lobe orifice appeared open. A bronchogram failed to fill the middle lobe bronchi.

This was thought to represent a typical middle lobe syndrome. Since prolonged conservative therapy had been of no help, surgical treatment was advised. At the time of surgery, the middle lobe was found to be atelectatic. Multiple calcified nodes were seen surrounding the middle lobe bronchus which had compressed it and caused the collapse. The middle lobe was removed. The postoperative course was uneventful. In the six months following surgery, the patient has completely regained her health. She has gained twenty pounds and no longer coughs.

BRONCHOLITHIASIS

A calcified hilar node may erode any portion of the tracheobronchial tree, but the most common site is the intermediate bronchus. Because fragments of these calcareous deposits may break off and be coughed up and expectorated, they have been called bronchial stones or broncholiths. Metastatic calcification due to metabolic abnormalities and senile calcification of bronchial cartilage may result in bronchiolithiasis. However, dystrophic calcification in a hilar node and its ultimate bronchial erosion is by far the most common etiologic factor.

In 1744, this condition was described by the Dutch physician Boerhaave (5). Only 142 cases had been reported in the world literature by 1954. This paucity of cases is by no means an indication of the true incidence of the disease. Substance is given this latter impression by the experience of Groves and Effler, who have reported their surgical experience in 27 cases accumulated over a perod of only a few years (6).

Erosion of the bronchus may come about by one of two basic mechanisms: (*a*) alternate elongation and shortening of the bronchus during respiration, and (*b*) infection and suppuration in the region of a previously located hilar calcification. This latter concept is not as soundly based on fact as the former and, indeed, may be spurious. If a broncholith is large enough to cause bronchial obstruction, the distal tracheobronchial tree may run the gamut of inflammatory change from simple bronchitis to bronchiectasis and lung abscess. Some observers have interpreted this situation to indicate that the calcific deposit is secondary to the inflammatory change (7). The unlikeliness of the validity of this concept is readily apparent.

The eroding mass presents on the endobronchial surface and in actuality represents a foreign body. It has been likened to an iceberg with the submerged portion of the berg analogous to the extraluminal portion of the stone. From time to time, fragments of the stony node may break off and suddenly present in the oropharynx after a paroxysm of coughing. Careful questioning will often elicit the history of expectoration of a gritty substance. At times the patient will save this substance and bring it to the physician for examination. During the course of erosion there may be recurrent hemoptysis. Chronic cough and symptoms due to a

distal inflammatory process are the usual findings. Repeated respiratory infections and areas of lobar or segmental consolidation visible on roentgenography may suggest the possibility of a bronchogenic carcinoma. Bronchiectasis of severe degree often develops in the distal tree because of partial proximal obstruction.

The initial step in arriving at a correct diagnosis is an awareness of the clinical entity. In the patient with roentgenographic evidence of hilar node calcification and symptoms and signs as described, there are several helpful diagnostic steps which may be taken. Bronchoscopy is of inestimable help. The broncholith may be seen impinging upon the bronchial lumen, resembling very closely a bronchogenic carcinoma. A biopsy can be obtained easily which on histologic examination discloses the presence of marked inflammatory change and calcareous substance. Bronchography is usually accomplished at the same time, and impingement of the bronchial lumen often may be nicely demonstrated. This study will also afford evaluation of the tracheobronchial tree distal to the broncholith with respect to the presence of bronchiectasis, since this is a necessary consideration in treating this ailment. Laminagraphy has been very helpful in assessing the over-all problem. Careful technique will afford an excellent view of the calcified node as it presses upon the bronchus.

The treatment of broncholithiasis is surgical (8). Some reports of bronchoscopic removal of the stone have been recorded, but this obviously is a technique fraught with danger and inherently inadequate. If the entire stone is removed via the bronchoscope—a virtual impossibility—it will leave a hole in the bronchus which is tantamount to a bronchopleural fistula. This complication, of course, may be a condition worse than the original disease process. Transpleural exploration offers the soundest, safest, and most effective form of therapy. If bronchiectasis has been demonstrated by the previous studies, excision of the involved lobe or segment and removal of the calcified node are indicated. Failure to remove the calcareous substance after lobectomy or segmentectomy may allow the bronchial stump to remain in contact with the hard node. This is an invitation to a persistent bronchopleural fistula. Closure of the fistula may depend on the removal of the calcific mass.

If the bronchial tree has not been irreparably damaged by bronchiectasis, it may be possible to excise the broncholith and allow the lung to remain intact. The resultant defect in the bronchus can be sutured directly and reinforced with a pedicle graft of pleura. This is the ideal form of treatment since it does not require the excision of lung tissue (9). However, in certain situations it may not be technically possible to remove the stone alone, and pulmonary tissue must be excised. It would seem, however, that an aggressive surgical attack early in the course of the illness will preclude the necessity for resection in many cases. In well-established cases when there is multiple node calcification, the actual surgical technique of lobectomy is often quite difficult as the nodes are intimately fixed to arteries, veins, and bronchi. Tissue cleavage plains are nonexistent and the dissection thereby becomes laborious and prolonged, but this does not necessarily preclude an excellent clinical result.

The problem presented by erosion of calcified node into the tracheobronchial tree is represented in figures 2, 3, and 4.

This patient, a 45-year-old white female, had been treated for asthma for years. She was chronically ill, thin, and had been subject to frequent paroxysms of coughing. She also complained of dysphagia. The pain appeared to be located in the midline at about the level of the fifth rib an-

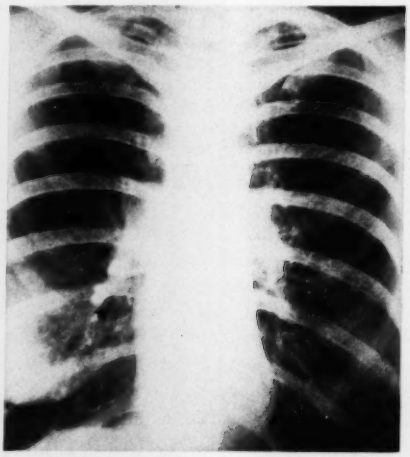

FIG. 2. Chest film of patient with scattered hilar calcification, severe cough, hemoptysis, and "asthmatic" history.

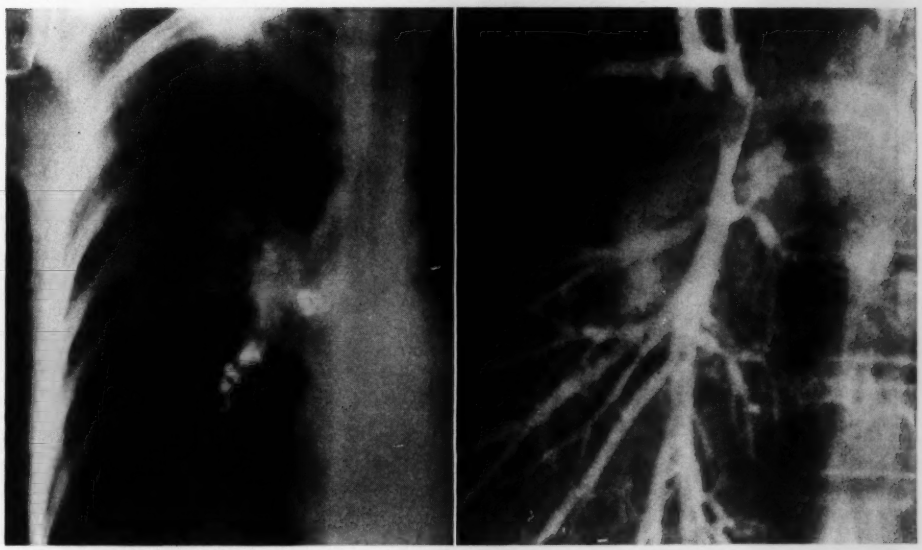

FIG. 3 (Left). Laminagram through right hilus showing bronchial impingement by calcified nodes.
FIG. 4 (Right). Bronchogram showing narrowing of bronchus due to calcified node. Complete recovery followed transpleural removal of stone and repair of bronchus.

teriorly. There had been two episodes of severe incapacitating pain in this region which resembled myocardial infarction. Occasionally there had been hemoptysis and on two instances the patient had coughed up some "gritty" particles. The few weeks prior to admission to the hospital had been associated with an increase in all symptomatology. She had been febrile and coughed repeatedly. A chest film disclosed a density in the region of the superior segment of the right lower lobe which some examiners interpreted as neoplasm. Bronchoscopy disclosed diffuse tracheobronchitis and narrowing of the intermediate bronchus. A mass "peeking" through the mucosa was noted which obscured the superior segmental orifice. A biopsy taken at this point was interpreted as showing chronic inflammatory change and calcareous substance. Bronchograms and laminagrams further delineated the broncholith and its relation to the bronchus.

The patient received antimicrobial therapy appropriate for the organism cultured from the tracheobronchial secretions. Intermittent positive pressure breathing therapy, high humidity, and other measures were instituted to improve the inflammatory component in this illness. Surgery was advised and carried out. At thoracotomy, the superior segment of the right lower lobe was collapsed and hard. A biopsy was taken which, on frozen section, revealed pneumonitis. A hard calcific mass was visualized which had eroded into the intermediate bronchus. Careful dissection was carried out and the calcified node removed. A diverticulum of the esophagus was found stuck to the calcified node. This was dissected free, amputated at its mouth, and the esophagus was closed in layers. After dissection, a moderately large hole in the posterior wall of the bronchus was left, as it had completely eroded this area. The bronchus was mobilized and sutures were placed in edges of the defect which was closed in a transverse fashion to avoid compressing the lumen. A pedicled graft of parietal pleura was fashioned and swung over the bronchus to reinforce this area. This was tacked to the bronchus with several interrupted sutures.

The patient's postoperative course was completely uncomplicated. In the next few months she rapidly gained weight and has lost her cough. Her "asthma" no longer troubles her. The dysphagia is completely relieved and she volunteered that swallowing is normal for the first time in many years.

A curious complication of a broncholith is illustrated in figure 5.

The patient was a 65-year-old man who had been admitted several times to the hospital with complaints of cough, wheezing, and febrile epi-

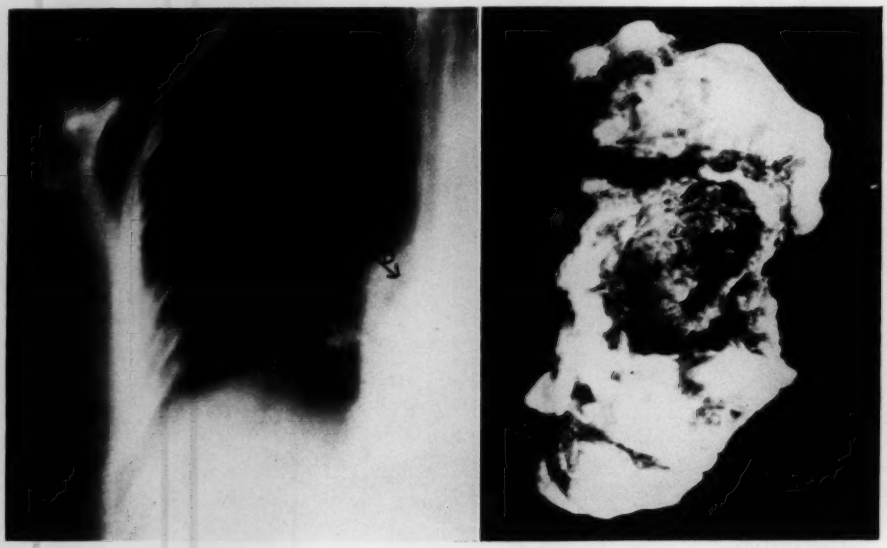

FIG. 5 (Left). Laminagram showing stone and bronchus. Previous resection of middle and right lower lobes. A bronchopleural fistula developed and closed after expectoration of broncholith.
FIG. 6 (Right). Expectorated broncholith (enlarged).

sodes. Bronchoscopy revealed the presence of polypoid tissue in the lower lobe bronchus. Much of this tissue was removed transbronchoscopically with the biopsy forceps, with considerable improvement. However, recurrence was prompt, as demonstrated by repeat bronchoscopy. He was readmitted with severe chest pain, elevation of temperature, and cough. A chest film disclosed apparent collapse of the right lower lobe. Thoracotomy was carried out several days later, and the middle and lower lobes were removed. Dissection was difficult because of the presence of many calcified peribronchial nodes. A bronchopleural fistula developed about ten days following surgery. A rib resection and insertion of a tube into the space was carried out to decompress the fistulous pocket. The fistula was extremely stubborn and, not until several months later—following a severe bout of coughing during which the broncholith pictured in figure 6 was expectorated—did it close.

This experience has stressed the importance of removing completely the calcareous substance surrounding a bronchial stump following pneumonectomy or lobectomy for any cause.

Esophageal Diverticulum

Traction diverticuli of the esophagus usually occur at the level of the bifurcation of the trachea and point anteriorly. These pockets which originate from traction by an inflamed lymph node are, like the calcified hilar node, given little consideration. Careful interrogation of the patient will often yield a history of dysphagia and recurrent severe chest pain. The latter may be intense enough to mimic myocardial infarction.

During the initial period of lymphadenitis, the surrounding tissues become fixed together. As the node becomes calcified, it may be stuck fast to a diverticulum on one front and to a bronchus on the other. Erosion of both of these visci may result in a free communication, in which event a bronchoesophageal fistula is established wherein the patient may cough following swallowing. Kidd and Christopherson, in 1951, wrote concerning the "chance" coexistence of broncholithiasis and bronchoesophageal fistula (10). This, the writers believe, was actually the clean-cut consummation of a single disease entity: hilar node inflammation and calcification. Credence is added to this concept by the report of Katz, who noted five instances of esophageal diverticuli in eighteen cases of middle lobe syndrome (11). This was the first time that the relation of these lesions was

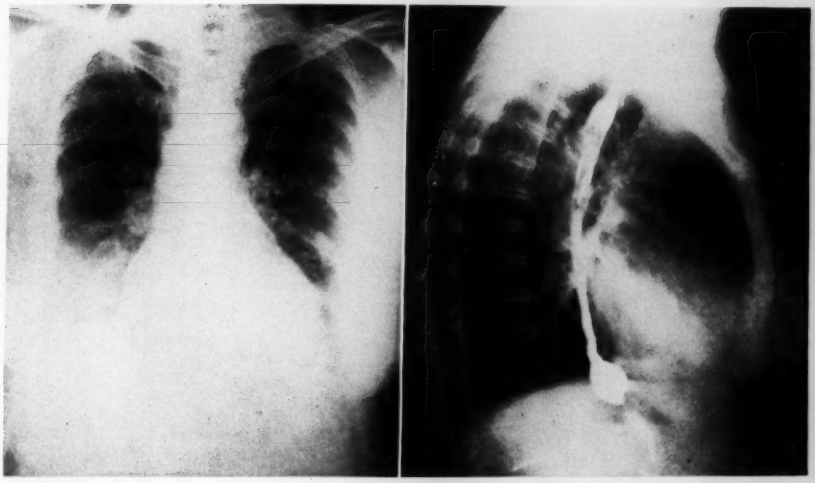

FIG. 7 (Left). Chest film of patient with middle lobe inflammation secondary to bronchoesophageal fistula. Hilar calcification is noted.

FIG. 8 (Right). Barium swallow demonstrating diverticulum and communication with middle lobe bronchus.

suggested. Both lesions were produced by node calcification.

A bronchoesophageal fistula, presumably the result of an eroding calcified node, is represented in figure 7.

The patient, a 67-year-old white female, had been well until eighteen months prior to admission to the hospital. A hacking cough began which was largely nonproductive. This was followed shortly thereafter by coughing up some "stony" material. Following this, she coughed in paroxysms after drinking or ingesting food. She had repeated febrile episodes and lost weight steadily. The symptoms continued to progress for more than a year. A chest film disclosed the presence of density in the region of the middle lobe. A barium swallow outlined a diverticulum and showed communication with the middle lobe bronchus (figure 8). A diagnosis of bronchoesophageal fistula was made and surgery was advised. At thoracotomy, an esophageal diverticulum was found bound to the middle lobe bronchus. This was dissected free, amputated, and the esophagus was closed in layers. The middle lobe was the site of a diffuse inflammatory process. Middle lobectomy was accomplished without incident. The patient made an uneventful recovery and has remained completely well since surgery.

It is presumed that the fistula developed from apposition of the diverticulum to the calcified node posteriorly and the middle lobe bronchus anteriorly. The stone eroded the bronchus and was expectorated. The many scattered hilar calcifications and the history lend substance to this hypothesis.

The detection of an esophageal diverticulum in the midportion of the esophagus is quite easily accomplished by fluoroscopy following a swallow of barium. It may prove helpful to study the patient in the prone position to allow the diverticulum the aid of gravitational force in filling. One swallow of barium may delineate the fistula and outline the bronchi in the case of a communication between the esophagus and tracheobronchial tree. Visualization of the diverticulum may be accomplished by esophagoscopy. The treatment of a symptomatic esophageal diverticulum is surgical. A transpleural approach with the dissection of the diverticulum is carried out. It is then resected and the wall of the esophagus approximated.

In several patients subjected to surgery for broncholithiasis, the writers have found an esophageal diverticulum bound to the offending calcified node. Resection of the diverticulum and removal of the mass have resulted in rather striking clinical improvement. On questioning these patients postoperatively, many annoying symptoms clearly due to the esophageal di-

verticulum were noted to have been eliminated. How easy it is to group the symptoms related to swallowing into a convenient compartment such as globus hystericus or neuromuscular dysfunction! These experiences suggest that a new look be taken at this lightly regarded lesion.

Superior Vena Caval Obstruction

Frank obstruction of the superior vena cava may occur by compression from calcified nodes. Again the pathogenesis of node calcification is similar. The superior cava is easily compressible, yet has little room for displacement. The clinical picture of caval occlusion is well known since it is seen frequently as a result of bronchogenic carcinoma. The veins of the head, neck, and upper extremities are dilated and the venous pressure is markedly elevated. A simple venogram, accomplished by injecting the right antecubital fossa with a radiopaque dye, will clearly demonstrate the site of caval obstruction. Often, on a plain chest film a calcific mass can be visualized in juxtaposition to the superior cava. The calcified node which is located in such a position to embarrass caval drainage also frequently endangers the trachea and right main stem bronchus. Irritation of the trachea has, in the writers' experience, caused such a distressing cough that the patient has sought medical help shortly after the onset of symptoms. Several of these patients have been observed who complained of cough on turning the head, presumably thereby increasing the impingement on the trachea.

The caval obstruction may be partial, as manifested by a slight rise in the venous pressure in the upper extremities. In these situations the mass can usually be dissected free of the surrounding tissues without great difficulty. It is urgently recommended that these lesions be excised before complete caval obstruction occurs. If the mass so intimately encircles the cava that resection of a portion of the vessel is necessary, it may have to be repaired by end-to-end anastomosis or the insertion of a graft. While the writers have had no occasion to graft for caval obstruction, it has been accomplished by others many times. Allansmith and Richards have reviewed this technique in a recent article (12).

Partial caval compression is represented in figure 9.

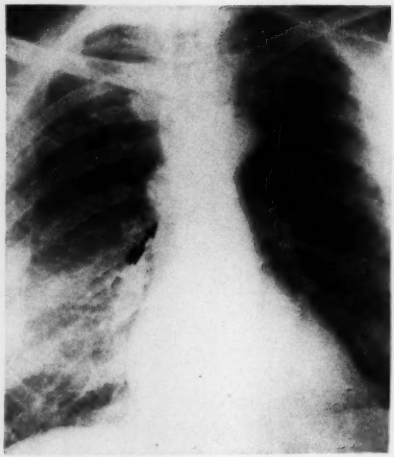

Fig. 9. Large calcified node compressing the superior vena cava and causing paroxysms of coughing. Relief of symptoms followed excision of the mass. Venous pressure returned to normal.

A 49-year-old male was admitted to the hospital with a history of frequent severe paroxysms of coughing. The coughing was often precipitated by change in position or movements necessary in his capacity as a freight handler. He coughed every morning while shaving his neck. He had been seen by many physicians without diagnosis or effectual treatment and had been advised, finally, to seek psychiatric help.

On physical examination, some prominence in the veins of the upper extremities was noted. Cough could also be precipitated by extension of the head. A chest film disclosed the presence of a calcific mass in juxtaposition to the trachea. A tracheogram clearly revealed erosion of the tracheal cartilages by this mass. It was thought that the entire symptom complex was due to tracheal compression and erosion and slight caval compression. Surgery was advised and accepted. At operation, a firm whitish mass, 3 cm. in diameter, was found which lay anterolateral to the trachea and in proximity to the superior vena cava. There was moderate compression of the cava. A large part of the mass extended below the carina, but sharp dissection of the fibrous pseudocapsule allowed complete removal of the mass.

The patient's recovery was uneventful and his formerly distressing symptomatology is no longer present.

In figures 10 through 13 are demonstrated involvement of the bronchus, esophagus, and superior vena cava, in sequential order. In figure 12, in addition to compromising the superior vena cava, the calcific mass has impinged upon the right main stem bronchial stump and produced a fistula. The original surgery should have included

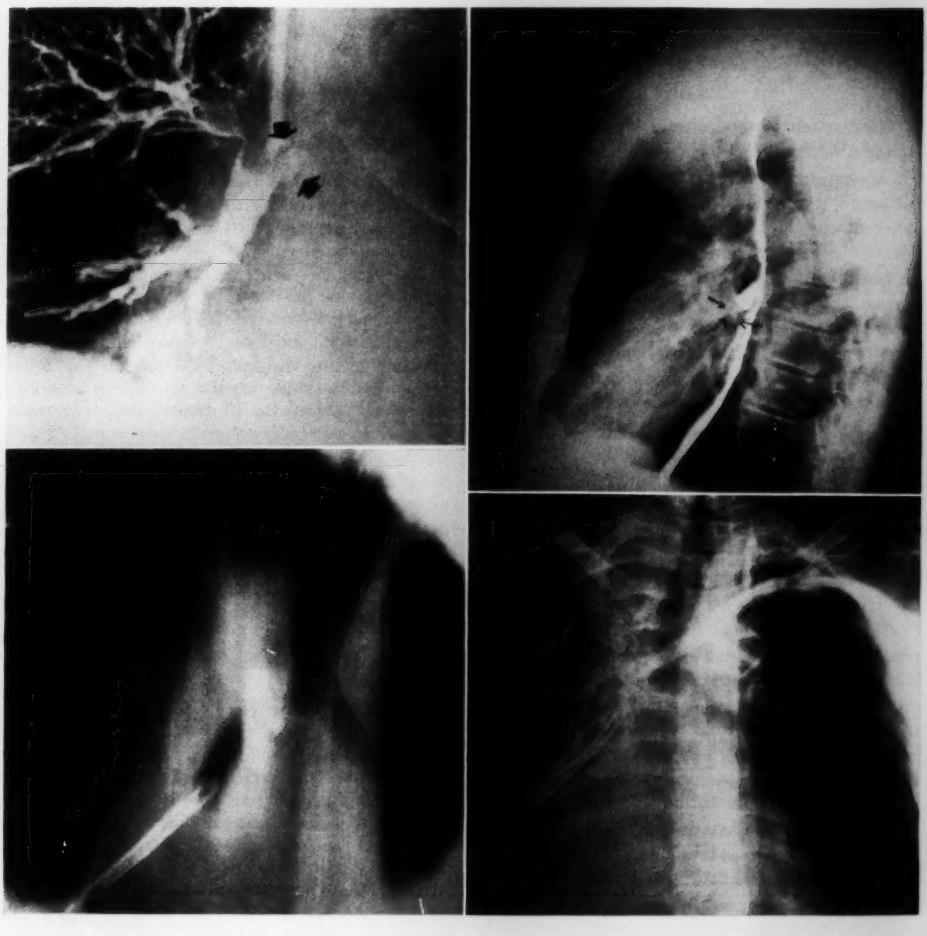

FIG. 10 (Top left). A 63-year-old white male with far advanced bronchiectasis of right lower lobe secondary to obstruction of bronchus by calcified node. Middle lobe bronchus not filled. This patient was advised to submit to surgical treatment, but refused.

FIG. 11 (Top right). Roentgenogram demonstrating esophageal diverticulum attached to calcified hilar node.

FIG. 12 (Lower left). Calcified node causing complete caval obstruction and bronchopleural fistula. Previous surgery precludes possibility of removal.

FIG. 13 (Lower right). Column of dye obstructed at the superior cava by calcified node.

excision of this material, which would have prevented the debility associated with a bronchopleural fistula in a pneumonectomized patient. In figure 13, a venogram shows obstruction at the caval level.

* * *

The writers believe that an unrealistic attitude of complacency exists concerning the calcified hilar node. An ostrich-like policy of "head in sand" fails to dispell the fact that hilar calcification can and frequently does produce significant disease. It is thought that clinical awareness of this potential as well as continued careful appraisal of the patient's complaints may disclose a relation between lymph node calcification and clinical state. With a free use of the available diagnostic tools, a valid conclusion may be

reached and effective, aggressive therapy instituted.

Summary

Calcification of mediastinal lymph nodes is a common occurrence. It is noted in patients after the appearance of certain granulomatous lesions. Although it had been commonly thought that tuberculosis was the most frequent cause, this is not necessarily true in certain localities where, on careful examination of calcified nodes, *Histoplasma capsulatum* has been found. The active lesion of histoplasmosis is characteristically the site of calcific deposition in its end stage. Whereas the inactivity of such a lesion is obvious, it presents, nonetheless, a potentially hazardous situation.

Irrespective of initial cause, the phenomena which may transpire depend upon the anatomic location of the calcified nodes. Nodes in contact with a bronchus may gradually erode its wall. In other instances the node may compress the bronchus and produce atelectasis. Traction diverticulum of the esophagus and compression of the superior vena cava are the remaining possible manifestations. The clinical picture with each lesion is clearly recognizable and in most cases can be satisfactorily managed. The patient with hilar node calcification should be studied with respect to the possible related clinical manifestations. A chronic cough or dysphagia should not be lightly regarded. Either of these symptoms may be due to calcified nodes. Special studies such as bronchography or laminagraphy may demonstrate the anatomic relation of node and bronchus, esophagus or vena cava.

A high degree of awareness must be maintained in order that effective therapy be instituted. The tendency in the past has been to overlook the pathogenic possibilities of calcified hilar nodes. This attitude is not justified by fact. As in many situations, early aggressive treatment will often preclude the development of the disastrous complications which are so often the result of prolonged conservative management.

Sumario

El Ganglio Hiliar Calcificado: Su Importancia y Asistencia (Reseña)

La calcificación de los ganglios linfáticos del mediastino es un acontecimiento común, notándose en los enfermos después de la aparición de ciertas lesiones granulomatosas. Aunque se había creído generalmente que la tuberculosis era la causa más frecuente, no es éste el caso forzosamente en ciertas localidades, en las que, al hacerse un examen detenido de los ganglios calcificados, se ha descubierto el *Histoplasma capsulatum*. La lesión activa de la histoplasmosis es típicamente el asiento de depósitos calcíferos en su período terminal. Aunque la inactividad de una de esas lesiones es manifiesta, crea, no obstante, una situación potencialmente peligrosa.

Independientemente de la causa inicial, los fenómenos que pueden sobrevenir dependen de la localización anatómica de los ganglios calcificados. Los ganglios en contacto con un bronquio pueden paulatinamente desgastar la pared. En otras ocasiones, el ganglio puede comprimir el bronquio y producir atelectasia. El divertículo por tracción del esófago y la compresión de la vena cava superior son las restantes manifestaciones posibles. El cuadro clínico de cada lesión es netamente reconocible y puede atenderse satisfactoriamente en la mayoría de los casos. El enfermo que tiene calcificación de los ganglios hiliares debe ser estudiado con respecto a las posibles manifestaciones clínicas relacionadas con el caso. No debe pasarse a la ligera una tos o disfagia crónica. Cualquiera de esos síntomas puede deberse a ganglios calcificados. Estudios especializados, tales como la broncografía o la laminografía, pueden revelar la relación anatómica del ganglio y del bronquio, esófago o vena cava.

A fin de aplicar una terapéutica eficaz, hay que mantener una vigilancia extremada. En el pasado, la tendencia ha sido hacia desatender las posibilidades patógenas de los ganglios hiliares calcificados. Esta actitud no está justificada por los hechos. Lo mismo que en otras muchas situaciones, el tratamiento osado temprano impedirá a menudo la aparición de las desastrosas complicaciones que son tan frecuentemente la consecuencia de la prolongada asistencia conservadora.

Resume

Ganglion calcifié du hile. Sa signification et conduite à tenir. Une revue

La calcification des ganglions lymphatiques médiastinaux est d'une fréquence banale. C'est noté chez les malades après l'apparition de certaines lésions granulomateuses. Bien qu'on ait pensé communément que la tuberculose en était la cause la plus fréquente, ce n'est pas nécessairement vraie dans certaines localités où, à l'examen minutieux des ganglions calcifiés, *Histoplasma capsulatum* a été trouvé. La lésion active de l'histoplasmose est d'une façon caractéristique le

siège de dépôt de calcium à son stade terminal. Bien que l'inactivité d'une telle lésion est évidente, elle présente néammoins une situation qui peut être dangereuse.

Sans compter la cause initiale, le phénomène qui peut en résulter dépend de la localisation anatomique des ganglions calcifiés. Des ganglions en contact avec une bronche peut l'éroder petit à petit. Dans d'autres circonstances, le ganglion peut comprimer la bronche et produire une atelectasie. D'autres manifestations possibles sont le diverticule de l'oesophage par traction et la compression de la veine cave supérieure. Le tableau clinique de chaque lésion est aisément reconnaissable et dans la plupart des cas peut être traité d'une façon satisfaisante. Le malade ayant des calcifications des ganglions du hile devrait être examiné en ayant dans l'esprit les manifestations cliniques possibles ci'dessus énonçées. Il ne faut pas sous-estimer une toux chronique et une dysphagie. L'un ou l'autre de ces symptômes peut être du à des ganglions calcifiés. Des études spéciales telles qu'une bronchographie ou une tomographie peuvent mettre en évidence la relation anatomique d'un ganglion et d'une bronche, de l'oesophage et de la veine cave.

Il faut garder un haut degré de suspiçion de façon à instituer une thérapeutique efficace. Dans le passé on a eu tendance à négliger les possibilités pathogèniques des ganglions hilaires calcifiés. Cette attitude n'est en fait pas justifiable. Comme dans beaucoup de situations, un traitement précoce agressif évitera souvent le développement de complications désastreuses qui sont si souvent le résultat d'une conduite conservatrice prolongée.

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A COMPARISON OF ISONIAZID–CYCLOSERINE WITH ISONIAZID–PAS IN THE THERAPY OF CAVITARY PULMONARY TUBERCULOSIS

X. A Report of the Veterans Administration–Armed Forces Cooperative Study[1, 2, 3, 4]

PATRICK B. STOREY

(Received for publication October 8, 1959)

Introduction

Cycloserine, D-4-amino-3-isoxazolidine, was discovered in 1954 (1, 2) and found to be a broad-spectrum antimicrobial with a relatively weak antituberculous effect *in vitro* and in experimental tuberculous infections (3–7). Various clinical studies have indicated that it possesses some therapeutic value in the management of human tuberculosis (8–10). A controlled clinical trial to compare its effect with that of isoniazid-PAS indicated that, when used alone in a dose of 1.0 gm. per day, it was decidedly less effective than isoniazid-PAS and considerably toxic (11, 12). Evidence has been presented that cycloserine at a dose of 0.5 gm. per day along with 300 mg. of isoniazid per day may be an effective therapeutic regimen (13, 14).

The Veterans Administration–Armed Forces Cooperative study group undertook a study of the clinical efficacy of an isoniazid–cycloserine regimen in the treatment of cavitary pulmonary tuberculosis. The purpose of the study was to determine whether this regimen was as effective as isoniazid-PAS and whether reduction of the dose of cycloserine to 0.5 gm. per day would eliminate or reduce its toxicity. To this end, the effect of the regimen isoniazid–cycloserine was compared with that of the regimen isoniazid–PAS when given to contemporaneously treated groups of patients with comparable disease.

Material and Methods

Fourteen Veterans Administration hospitals participated in this study, each contributing cases to both regimens involved. The study was controlled by the method of random allocation of regimens based, at first, upon the hospital registry number system and subsequently upon a sealed envelope system of allocation. Approximately half of the cases were randomized by each method.

Only patients who had cavitary disease, whose sputum contained tubercle bacilli, and who had not previously received antimicrobial therapy for tuberculosis were included in the study. Patients with histories of convulsions or psychosis, with marked impairment of renal function, or with bromsulphothalein retention of more than 7 per cent were excluded from the study.

There were actually two separate simultaneous studies carried out, one addressed to the comparison of two regimens, isoniazid–cycloserine and isoniazid–PAS; and the other to a comparison among three regimens, isoniazid–cycloserine, isoniazid–PAS, and isoniazid–pyrazinamide. The patients from both studies who were treated with isoniazid–cycloserine or isoniazid–PAS have been combined to yield the data of the present report. However, patients who were treated with isoniazid–pyrazinamide will not be considered further in this report.

The dose of cycloserine[5] employed was 0.5 gm. per day, in a 250-mg. dose given every twelve hours. The dose of isoniazid was 300 mg. per day, given as 100 mg. three times daily. The dose of PAS was 12 gm. of the acid or its equivalent per day, 4 gm. three times daily.

The background data of the patients admitted to study are shown in table 1. The extent of disease at the time of admission to the study is shown in both the standard National Tuberculosis Association terminology and in the Veterans Administration–Armed Forces system which is based on the size and the number of cavities present. It will be noted that the patients on the two regimens were quite similar, but the section on extent of disease deserves some comment. There were slightly more far advanced cases and slightly more cases of large single cavities in the isoniazid–PAS group but, on the other hand, there were more patients with

[1] From the Veterans Administration Hospital, Baltimore, Maryland.

[2] This report was prepared by the writer at the request of the Executive Committee of the Conference on Chemotherapy of Tuberculosis, Veterans Administration–Armed Forces.

[3] Preliminary presentations of this report were made at the Seventeenth and Eighteenth Veterans Administration–Armed Forces Conferences on the Chemotherapy of Tuberculosis in 1958 and 1959.

[4] The names of the hospitals which have participated in this cooperative study are listed under "Acknowledgments" at the end of the article.

[5] The cycloserine used in this study was generously supplied as Seromycin by Eli Lilly and Company, Indianapolis, Indiana.

TABLE 1

BACKGROUND FACTORS AT START OF CHEMOTHERAPY

Background Factors	Regimen			
	Isoniazid-PAS		Isoniazid-Cycloserine	
	Number	Per Cent	Number	Per Cent
Total cases	368		364	
Age: Total known	367	100	363	100
Less than 40	180	49	175	47
40 and older	187	51	188	53
Race: Total known	348	100	350	100
White	244	70	240	68
Negro	94	27	107	31
Other	10	3	3	1
Lung involvement: Total known	367	100	363	100
Unilateral	110	30	121	33
Bilateral	257	70	242	67
Extent of disease, group, and cavitation:				
Total known	365	100	362	100
Moderately advanced, total	128	35	134	37
Group II (single cavity)*	78	21	90	25
A (2.0 cm. or less)	52	14	67	19
B (2.5–3.5 cm.)	26	7	22	6
C (4.0 cm. or more)	0	0	0	0
Group III (multiple cavities)	43	12	41	11
A (2.0 cm. or less)	35	10	26	7
B (2.5–3.5 cm.)	8	2	13	4
C (4.0 cm. or more)	0	0	0	0
Far advanced, total	237	65	228	63
Group II (single cavity)	62	17	44	12
A (2.0 cm. or less)	20	5	12	3
B (2.5–3.5 cm.)	12	3	11	3
C (4.0 cm. or more)	28	8	21	6
Group III (multiple cavities)	166	45	177	49
A (2.0 cm. or less)	43	12	36	10
B (2.5–3.5 cm.)	56	15	57	16
C (4.0 cm. or more)	67	18	81	22

* In the Veterans Administration–Armed Forces system of classification, Group I refers to no cavity Group II, to single cavity; and Group III, to multiple cavities. Noncavitary cases were excluded by the terms of the protocol.

multiple cavities and more with multiple large cavities in the isoniazid–cycloserine-treated group. As it turns out, however, these differences did not exert significant influence on the results of the study.

The status of chemotherapy of all cases and the reasons for loss of cases from the study are shown in table 2. It will be noted from the table that the study was divided into two four-month observation periods. There were 368 isoniazid–PAS-treated cases observed and reported during the first four months, compared with 364 isoniazid–cycloserine cases. There were 280 isoniazid–PAS and 284 isoniazid–cycloserine cases during the second four-month period on which reports were received.

RESULTS

Roentgenographic Change

The total experience with respect to roentgenographic change is shown in table 3. The films were evaluated and reported by the staff of the participating hospitals.

In terms of moderate or marked improvement, there was little inter-regimen difference for either observation period for the total number of cases.

TABLE 2
STATUS OF CHEMOTHERAPY

	Months of Observation							
	0–4 Months				5–8 Months			
	Isoniazid-PAS		Isoniazid-Cycloserine		Isoniazid-PAS		Isoniazid-Cycloserine	
	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Total cases observed	368	100	364	100	280	100	284	100
Chemotherapy continuing, protocol	294	79	298	81	198	71	169	60
Chemotherapy changed, total	40	11	38	11	42	15	76	26
Toxicity to: isoniazid	1	a*	2	1	0	0	0	0
PAS	29	8	0	0	8	3	0	0
cycloserine	0	0	14	4	0	0	8	3
not specified	1	a	1	a	1	a	2	1
Resistance to: isoniazid	0	0	1	a	1	a	4	1
PAS	0	0	0	0	0	0	0	0
cycloserine	0	0	1	a	0	0	3	1
not specified	0	0	0	0	0	0	2	1
Worsening by: roentgenography	0	0	10	3	0	0	5	2
bacteriologically	0	0	1	a	0	0	0	0
both	1	a	0	0	0	0	1	a
not specified	0	0	2	1	0	0	0	0
No improvement: roentgenographic	0	0	0	0	1	a	1	0
bacteriologic	0	0	0	0	3	1	4	1
both	0	0	1	a	1	a	1	a
not specified	1	a	1	a	0	0	6	2
Discharge or transfer	0	0	0	0	1	a	8	4
Surgery	1	a	2	1	14	5	13	5
Other, not further specified	6	2	2	1	12	4	16	5
Chemotherapy stopped, total	34	10	28	8	40	14	39	14
Maximal benefit, or completion of study protocol	0	0	0	0	0	0	4	1
Death from any cause	7	2	3	1	3	1	0	0
Premature discharge	25	7	25	7	35	12	34	12
Other, not otherwise specified	2	1	0	0	2	1	1	a

*a Less than 0.5 per cent.

This table accounts for all patients who entered the study and shows the reason for discontinuation or change of therapy when it occurred.

However, at the four-month observation point more of the patients with large cavities who were treated with isoniazid–PAS showed substantial improvement (66 per cent of 90 cases) than those treated with isoniazid–cycloserine (57 per cent of 98 cases).

In the Veterans Administration studies, unfavorable roentgenographic progression during treatment is shown in two different ways, which are not always complementary. If an unfavorable change occurs on any film during treatment, it is recorded as such separately, regardless of the net or over-all change by the end of the observation period as compared with the pretreatment film. Such observations are recorded under "Unfavorable Roentgenographic Change" as in table 3. On the other hand, at the completion of each period of observation, the over-all roentgenographic change is indicated either in terms of betterment, failure to change, or worsening as compared with the pretreatment film. These observations, if untoward, are recorded as "worse" in table 3. It is possible that a particular patient may appear in both groups, but this is not necessarily the case. In order to find out how many patients actually showed "unfavorable progres-

TABLE 3
ROENTGENOGRAPHIC CHANGE

	Months of Observation							
	0–4 Months				5–8 Months			
	Isoniazid-PAS		Isoniazid-Cycloserine		Isoniazid-PAS		Isoniazid-Cycloserine	
	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Total cases observed	349	100	346	100	263	100	268	100
Marked or moderate improvement	207	59	196	57	197	74	203	76
Slight improvement	97	28	95	27	41	16	48	18
No change	41	12	40	12	25	10	9	3
Worse	4	1	15	4	0	0	8	3
Cavity, 2.0 cm. or less, total cases	144	100	138	100	116	100	116	100
Marked or moderate improvement	84	59	76	55	85	73	82	71
Slight improvement	42	29	41	30	16	14	24	21
No change	18	12	17	12	15	13	5	4
Worse	0	0	4	3	0	0	5	4
Cavity, 2.5–3.5 cm., total cases	104	100	100	100	73	100	77	100
Marked or moderate improvement	62	59	59	59	54	74	64	83
Slight improvement	31	30	25	25	12	16	12	16
No change	7	7	13	13	7	10	0	0
Worse	4	4	3	3	0	0	1	1
Cavity, 4.0 cm. or more, total cases	90	100	98	100	66	100	67	100
Marked or moderate improvement	59	66	56	57	55	83	53	80
Slight improvement	19	21	26	27	9	14	11	16
No change	12	13	9	9	2	3	1	1
Worse	0	0	7	7	0	0	2	3
Unfavorable roentgenographic change								
Total cases observed	368	100	364	100	280	100	284	100
Occurred	5	1	17	5	2	1	7	2
Did not occur	363	99	347	95	278	99	277	98

sion roentgenographically," all patients reported as having shown worsening or unfavorable roentgenographic change were identified and grouped as shown in table 4. There were 5 patients among those treated with isoniazid-PAS who showed roentgenographic progression of disease during the first four months of treatment. There were 2 such patients during the fifth to eighth months. The incidence of roentgenographic progression of disease was 2 per cent in this group. Among the isoniazid–cycloserine-treated patients, there were 18 in the first four months who showed such unfavorable change and 6 additional patients during the fifth to eighth months, for a total of 24 instances. The incidence of extension of disease in the isoniazid–cycloserine group was 7 per cent.

Treatment Failures

A favorable response to treatment for pulmonary tuberculosis is ordinarily expressed in terms of symptomatic and roentgenographic improvement, diminution of cavity size or closure of cavities, and reversal of infectiousness. When one or more of these events fail to materialize, or evidence appears of unfavorable progression of the disease, there will be a tendency on the part of the attending physician to alter the chemotherapy either by a change in dose of the drugs in use or by substitution of other drugs. In the

TABLE 4

UNFAVORABLE PROGRESSION OF DISEASE ROENTGENOGRAPHICALLY

	0–4 Months		5–8 Months	
	Isoniazid-PAS	Isoniazid-Cycloserine	Isoniazid-PAS	Isoniazid-Cycloserine
Roentgenographic worsening	4	13	0	6
Unfavorable roentgenographic change	1	5	2	0
Total roentgenographic progression	5	18	2	6

This table is constructed from the data of table 3 to show the total number of patients in each regimen who showed roentgenographic extension of disease. It differs from table 3 in that the same patient does not appear in both categories of "progression."

Veterans Administration cooperative studies, such patients are listed as lost to the study by virtue of the need for re-treatment.

Reference to table 2 will show that this happened with both drug regimens. For the patients treated with isoniazid-PAS, treatment had to be changed because of worsening or failure of 2 patients to improve during the first four months, and of 6 patients during the fifth to eighth months, a total of 8 patients during the period of observation. The incidence of treatment failure in this group was 2 per cent.

In the isoniazid–cycloserine group, treatment was changed in 17 cases during the first four months and in 27 during the fifth to eighth months, a total of 44 cases or 12 per cent of the 364 patients who started treatment with this regimen. Of these 44 patients, 19 were removed from the study because of worsening under treatment and 25 because of failure to improve (14 patients) or the appearance of drug resistance (11 patients). Included in this group was one patient who developed signs of meningitis and was found to have tuberculous meningitis after one month of treatment with isoniazid–cycloserine.

Cavity Closure

The incidence of cavity closure for all cases regardless of cavity size is calculated by the life-table method and shown in figure 1. There is a lesser incidence of cavity closure at six and at eight months of isoniazid–cycloserine therapy than there was with isoniazid–PAS (28 per cent versus 31 per cent and 39 per cent versus 44 per cent).

The percentage incidence of cavity closure related to cavity size at the start of treatment is shown in figure 2. It may also be noted that the small difference between the regimens is present regardless of cavity size.

"Sputum Conversion"

In all previous reports from the Veterans Administration–Armed Forces study group, the word "conversion" has been used to indicate reversal of infectiousness and has been defined as the absence from the bronchial secretions of culturable bacilli and acid-fast bacilli on microscopy for three consecutive examinations at least one month apart. This is a strict definition and leads to some difficulties which may not be appreciated by those who use the monthly observation system. Three problems in particular might be mentioned:

1. The patient is recorded as not having achieved "conversion" until the third of these cultures is reported. Once this point is reached, however, the "conversion" is backdated to the first culture negative for tubercle bacilli in the series.

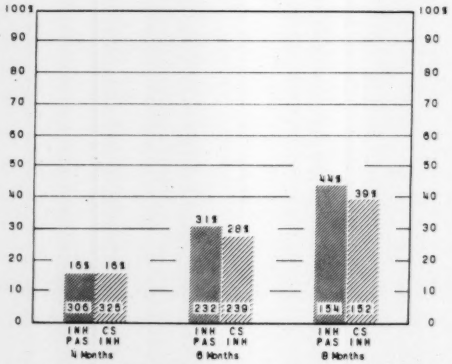

FIG. 1. Cavity closure of all cavities regardless of size. The percentage incidence of cavity closure is shown at the top of the bars and is calculated by the life-table method. The figures set within the bars represent the number of patients still at risk at each period of observation, i.e., those who had obtained cavity closure are excluded. INH = isoniazid; CS = cycloserine.

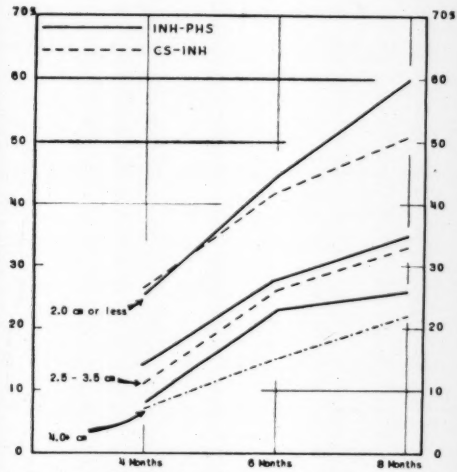

FIG. 2. Cavity closure related to cavity size. INH = isoniazid; CS = cycloserine.

2. Should a patient be lost to study after his secretions have become negative for tubercle bacilli but before he has been negative for three months, he cannot be counted as having "converted" his sputum.

3. The group of patients who will be lost to study for any reason over a three-month period will necessarily be larger than any number lost in a one-month period.

These factors will all tend to produce a seemingly poor bacteriologic response to therapy, since the total group is divided into those who have achieved noninfectious status and those who have not. In the present report, therefore, the bacteriologic results are presented both as they were in previous studies and also in the more conventional way of single-specimen observations.

The rate of attainment of noninfectious status in all cases regardless of cavity size is shown in figure 3. At all points of observation there is a small difference in favor of the isoniazid–PAS regimen.

The bacteriologic results expressed as per cent of negative observations of the total in whom a result was reported at four and at five to eight months of treatment are shown in figure 4. Patients were counted as "positive" if tubercle bacilli were found by either microscopy or culture. This again is an important point to make because some studies report only cultural results. At the five- to eight-month point there were 13 of the isoniazid–PAS patients positive for tubercle bacilli only by microscopy (5 per cent) and 15 of the isoniazid-cycloserine patients (6 per cent) were in the same state. Had only the cultural results been considered, the reversal of infectiousness percentage would have been higher, viz., 83 per cent for the isoniazid-PAS group and 74 per cent for the isoniazid-cycloserine group. In any case, there was a considerable difference between the two regimens, isoniazid-PAS showing a 10 per cent greater incidence of reversal of infectiousness at five to eight months of treatment.

Bacterial Resistance

The susceptibility of the pretreatment strains of tubercle bacilli to all three drugs employed was determined at each participating hospital and is shown in table 5. The specimens tested were almost uniformly susceptible to isoniazid and PAS, those not completely susceptible showing only partial growth in concentrations of 1 γ per ml. for isoniazid and 10 γ per ml. for PAS. The pretreatment strains from 98 per cent of the isoniazid–PAS cases showed no growth in any isoniazid-containing tube and the same was true for 92 per cent of the isoniazid-cycloserine cases. Moreover, 98 per cent of the strains from the isoniazid-PAS group showed no growth in any PAS-containing tube. Oddly enough, there was a higher frequency of partial growth in the 1 γ of isoniazid tube among the isoniazid–cycloserine cases (8 per cent) than among the isoniazid-PAS cases (2 per cent). Of those patients whose organisms were tested for susceptibility to cycloserine, 79 per cent of the patient strains were completely inhibited by 20 γ of cycloserine per ml., 14 per cent showed partial growth in 20 γ, and 7 per cent showed a higher order of resistance, 5 per cent showing at least some growth in 40 γ of cycloserine per ml.

Patients whose organisms showed greater resistance than was indicated by partial growth in the lower concentrations of the test drugs were not accepted for study and do not appear in table 5. In order to be sure that the pretreatment presence of partial resistance to 1 γ of isoniazid per ml. or to 20 γ of cycloserine per ml. did not influence the bacteriologic result, all such patients were identified and their response to treatment was separately checked. They did not influence the results of the study either in terms of persistent infectiousness of the sputum or as

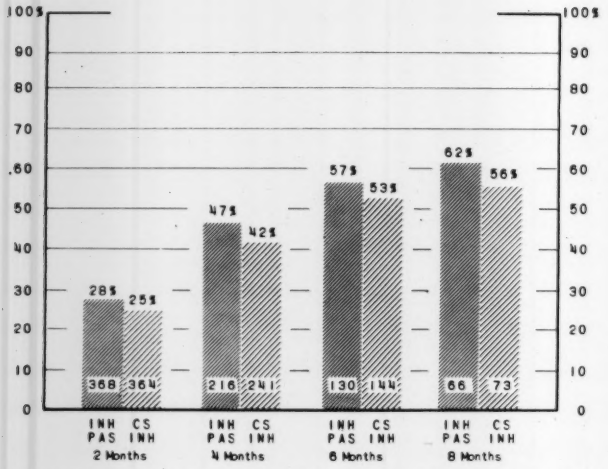

FIG. 3. Reversal of infectiousness. The percentage of patients whose sputum and/or gastric contents became noninfectious is shown at the top of the bars. The life-table method of calculation was used, and the figures set within the bars represent the number of patients still at risk at each period of observation. INH = isoniazid; CS = cycloserine.

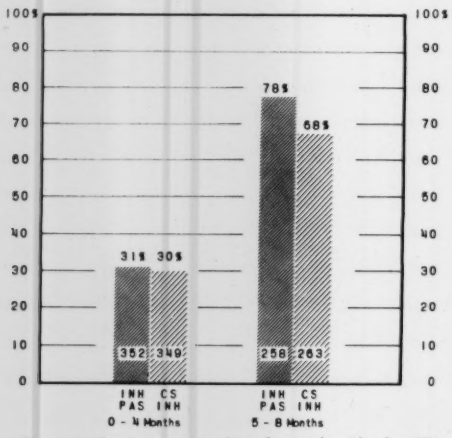

FIG. 4. Sputum negative for tubercle bacilli. The percentage of patients with secretions negative for tubercle bacilli are shown at the top of the bars. The total number of patients observed are set within the bars. INH = isoniazid; CS = cycloserine.

contributors to the group of patients eventually found to have tubercle bacilli resistant to isoniazid or cycloserine.

The emergence of organisms resistant to isoniazid in those patients whose sputum remained infectious and whose strains were tested is shown in table 6. In table 7 are shown the total cases observed, the incidence of infectious sputum, the number of those with tubercle bacilli in the sputum who were not tested for drug susceptibility, and the percentage incidence in the total study group of strains of tubercle bacilli resistant to isoniazid.

Several levels of isoniazid resistance may be defined from table 6. If any growth in 1 γ or more of isoniazid per ml. is defined as resistant, for example, then 20.5 per cent of the strains cultured at three to four months from patients (35 of 167 tested) under treatment with isoniazid-cycloserine contained isoniazid-resistant organisms. Only 7 per cent (10 of 146 patients tested) from the isoniazid-PAS group had such strains. At the five- to eight-month point, 48 per cent of the cultures from the isoniazid–cycloserine group (28 of 58 patients) contained resistant clones, and 36 per cent of the isoniazid–PAS-treated patients (14 of 39) who were still infectious were excreting such strains. Bacterial resistance, then, appeared much more quickly with isoniazid–cycloserine and in considerably greater incidence.

The appearance of resistance to cycloserine is shown in table 8. If the cycloserine resistance of a patient-strain of tubercle bacilli be defined as growth in 20 γ of cycloserine per ml., the inci-

TABLE 5

PRETREATMENT DRUG SUSCEPTIBILITY

	Isoniazid				PAS				Cycloserine		
	Isoniazid-PAS		Isoniazid-Cycloserine			Isoniazid-PAS			Isoniazid-Cycloserine		
	Number	Per Cent	Number	Per Cent		Number	Per Cent		Number	Per Cent
Total patient-strains tested	280		277			286			149	
Susceptible	275	98	256	92		279	98		118	79
Resistant	5	2	21	8		7	2		31	21
Partial growth, 1 γ	5	2	21	8	10 γ	7	2	20 γ	21	14
Equal growth in 1; none in 5	0		0		10–100	0		20–40	3	
Partial growth, 1 and 5 γ	0		0		10–100	0		20–40	4	
Equal growth, 1; partial, 5	0		0		10–100	0		20–40	2	
Equal growth, 5 γ	0		0		100	0		40	1	
Not tested	84		81			78			209	

The total drug-susceptibility observations obtained prior to start of treatment are shown with the definition of what is meant by resistance to each drug. The strains of tubercle bacilli from almost half of the patients treated with isoniazid-cycloserine were tested for cycloserine susceptibility.

TABLE 6

RESISTANCE TO ISONIAZID

	3–4 Months				5–8 Months			
	Isoniazid-PAS		Isoniazid-Cycloserine		Isoniazid-PAS		Isoniazid-Cycloserine	
	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Total patient-strains tested	146		167		39		58	
Susceptible	136	93	132	79.5	25	64	30	52
Resistant	10	7	35	20.5	14	36	28	48
Partial growth 1 γ	5	3.5	16	9.5	7	8	6	11
Equal growth 1 γ; 0–5 γ			4	2.5			1	0.5
Partial growth, 1 and 5 γ	3	2	6	3.5	2	5	6	11
Equal-1; partial-5 γ			7	4.0	3	8	7	12
Equal growth, 5 γ	2	1.5	2	1	2	5	8	13.5

Resistance is defined as any growth in the presence of isoniazid in the concentrations tested. Degrees of resistance are as shown. The percentage incidence of resistance shown is based on the number of patients whose strains of tubercle bacilli were actually tested. The incidence of emergence of resistance to isoniazid in the entire treatment group is shown in table 7.

dence of such strains at three to four months was 26 per cent and at five to eight months was 30 per cent, which is not greatly higher than the pretreatment figure of 21 per cent. If cycloserine resistance is defined as equal growth in 20 γ per ml., then the incidence of such patient-strains was 10 per cent at three to four months and 20 per cent at five to eight months, compared with 7 per cent showing such resistance before start of treatment. This may represent some shift in degree of resistance to cycloserine.

The emergence of resistance to PAS is shown in table 9. At three to four months of treatment, 91 per cent of patients whose secretions were infectious yielded organisms completely susceptible to 10 γ of PAS. Of the 9 per cent whose strains showed drug resistance, in the majority (9 cases) the strain showed only partial growth in 10 γ, and in 4 cases the strain showed either equal growth in 10 γ or some growth in 100 γ. The appearance of resistance to PAS among the 35 patients still infectious and with strains tested

TABLE 7
RESISTANCE TO ISONIAZID

	Months of Observation							
	0–4 Months				5–8 Months			
	Isoniazid-PAS		Isoniazid-Cycloserine		Isoniazid-PAS		Isoniazid-Cycloserine	
	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Total cases observed	368	100	364	100	280	100	284	100
Culture negative for *M. tuberculosis**	118	32	113	31	209	75	185	65
Culture positive for *M. tuberculosis*	146	40	167	46	39	14	58	21
No growth	136	38	132	37	25	9	30	11
Partial growth, 1 γ; none in 5 γ	5	1	16	4	7	2.5	6	2
Equal growth in 1 γ and/or any growth in 5 γ	5	1	19	5	7	2.5	22	8
Unknown: culture and/or susceptibility	104	28	84	23	32	11	41	14

* Acid-fast bacilli may be present on microscopy.

This table accounts for all patients observed and expresses the percentages in terms of the entire study group, rather than only those in whom susceptibility studies were available.

TABLE 8
RESISTANCE TO CYCLOSERINE

	3–4 Months		5–8 Months	
	Number	Per Cent	Number	Per Cent
Total patient-strains studied	119		40	
Susceptible	88	74	28	70
Resistant	31	26	12	30
Partial growth, 20 γ	19	16	4	10
Equal growth, 20 γ	12	10	8	20

Resistance is defined as any growth of tubercle bacilli in the presence of cycloserine in the concentrations tested. Degrees of resistance are as shown.

for PAS resistance at five to eight months was nil, only one patient-strain showing partial growth in 10 γ. In essence, the occurrence of resistance to PAS, as measured in this study, was a rare event. This was also true of cycloserine resistance. The situation was quite different with isoniazid, to which the appearance of resistance was relatively frequent in both series, and especially in the isoniazid–cycloserine-treated patients.

Drug Toxicity

In the isoniazid-PAS group, 30 patients were removed from study in the first four months because of symptoms of drug toxicity, and 8 were lost in the next four months. The total was 38 or 11 per cent of those who started treatment. The great majority of the toxic reactions were gastrointestinal intolerance to PAS, but there were also 6 instances of drug eruption, two of drug fever, and one death from acute yellow atrophy of the liver. A brief description of this case follows:

> . . . On the 36th day of treatment PAS was stopped because of a complaint of nausea with some fever, to 99.6° four days previously and to 101.2° the preceding day or 35th day of treatment. On the day following discontinuation of PAS a maculopapular rash became apparent and isoniazid was also discontinued. Patient developed a sore throat with tiny vesicles. The liver was palpable 3 cms. below the right costal margin and non-tender. Three days after stopping PAS he was still febrile. Jaundice was first noted eight days after stopping treatment, with clay-colored stools and dark urine. His course thereafter was fulminating, with death two days after the appearance of jaundice. Autopsy showed severe liver necrosis. The diagnosis was acute yellow atrophy.

Of the isoniazid–cycloserine-treated patients, 16 were removed in the first four months because of drug toxicity and 8 in the second four months, a total of 24 cases, or 7 per cent. These reactions differed from the isoniazid-PAS reactions in that

TABLE 9
APPEARANCE OF RESISTANCE TO PAS

	3–4 Months		5–8 Months	
	Number	Per Cent	Number	Per Cent
Total patient-strains studied	142		35	
Susceptible	129	91	34	97
Resistant	13	9	1	
Partial growth, 10 γ	9	6	1	
Equal growth, 10 γ	4	3	0	

Resistance is defined as any growth in the presence of PAS in the concentrations tested. Degrees of resistance are as shown.

they involved the central nervous system rather than the gastrointestinal tract. The reactions included personality, mental, and neurologic changes in 13 patients, drug eruptions in 2 (both due to cycloserine), optic neuritis in one, and convulsive seizure in one patient. The convulsive seizure was described as follows:

> . . . Patient complained of dizziness on the 149th day of treatment. On the 153rd day he suffered a short convulsive episode at 10 p.m. Cycloserine was discontinued. The patient left AWOL the following day. Information from relatives indicated that he had no further difficulty.

DISCUSSION

There are now many drug regimens available for the treatment of tuberculosis, certain of which are very effective in perhaps the majority of cases to be treated. The purpose of the controlled clinical trials conducted by the Veterans Administration–Armed Forces, the United States Public Health Service, and the Medical Research Council of Great Britain has been to try to assign some priority of effectiveness among these regimens, giving due consideration to the many factors involved, such as mode of administration, toxicity, clinical and roentgenographic improvement, reversal of infectiousness, and avoidance of emergence of drug-resistant strains of tubercle bacilli.

All of these studies have sought to eliminate bias by the method of concurrent study (with random selection) of at least two regimens, each hospital in the cooperating system using random selection for both regimens, so that all other variables that might be operative at different hospitals might be equally distributed between the two drug regimens.

Of the drug regimens tested so far by the Veterans Administration–Armed Forces, the double drug regimen of isoniazid–PAS in the now standard dose of 300 mg. of isoniazid and 12 gm. of PAS per day has always proved at least equal, if not superior, to any other regimen. Indeed, in spite of some theoretic considerations involved, it has not yet been possible to prove any advantage for either the addition of daily streptomycin to isoniazid–PAS (15) or the increasing of the dose of isoniazid some fourfold to 16 mg. per kg. (16).

Isoniazid–PAS has the marked advantage of oral administration, but does have the disadvantage that substantial numbers of patients cannot tolerate the PAS (11 per cent in the present series), principally because of its toxic effect on the gastrointestinal tract. It is also true that there will be 20 per cent or more patients who will not achieve as good a result as might reasonably be expected.

The appearance, therefore, of another orally administered agent is an exciting one and, when cycloserine was first reported to have striking therapeutic effect against pulmonary tuberculosis (8), it was accepted for controlled clinical study. The first controlled study of its relative clinical value demonstrated that, given alone in a dose of 1 gm. per day, it was decidedly inferior to isoniazid–PAS in all measurable respects and was quite likely at that dose to produce convulsions (11, 12). The dose was accordingly reduced, and the present study of the combination of cycloserine with isoniazid was undertaken.

Whereas the inferiority of cycloserine alone to isoniazid–PAS was easily demonstrated, once the remarkably potent isoniazid was added the difference between the two regimens might be expected to be relatively small and consequently less easy to demonstrate. This proved true, as indicated by the results of this study, but these results consistently favor isoniazid–PAS over isoniazid–cycloserine in a manner that leaves little room for doubt.

The question is invariably asked in such a study whether a test regimen has been shown to be clinically useful, even if it is somewhat inferior to a control regimen. Ultimately, in a sense, a

study such as this and the multiple controlled studies already done must provide this answer. But the provision of this answer is not the primary function of the controlled clinical study. Rather is this answer really a function of many variables. Undoubtedly, in many patients the isoniazid–cycloserine regimen will yield optimal results, as may streptomycin-PAS, isoniazid–streptomycin, or isoniazid alone. But whether isoniazid–cycloserine, in the doses used, would yield better results in a controlled clinical study of a number of cases than would isoniazid alone would be indeed a moot point, not susceptible to answer from the data of the present study.

Summary

The data from the Veterans Administration–Armed Forces cooperative study of the clinical effectiveness of the two drugs cycloserine and isoniazid used together have been analyzed. The efficacy of this regimen was measured against that of isoniazid and para-aminosalicylic acid (PAS) given concurrently to a control group of patients with cavitary tuberculosis in fourteen cooperating hospitals.

In several respects, results in the isoniazid–cycloserine-treated group tended to show that this regimen is less efficacious than isoniazid–PAS. These results are: A higher incidence of roentgenographic evidence of progression of the disease, 7 per cent as compared with an incidence of 2 per cent among the control group; a much higher incidence of clinical failure to respond satisfactorily to treatment, which necessitated change of drug regimen in 12 per cent of the cases as compared with 2 per cent in the isoniazid–PAS–treated group; a lesser incidence of cavity closure; a lower incidence of appearance of bacteriologic negativity and bacteriologic "conversion" of the bronchopulmonary secretions; a more rapid emergence of organism resistance to isoniazid with a greater over-all incidence of resistance to isoniazid.

Although (in general) well tolerated, the regimen is not without its own toxicity as evidenced by the occurrence of one convulsive seizure, one instance of optic neuritis, 3 hypersensitivity reactions, and perhaps 13 instances of mental aberration or behavioral change. In the isoniazid–PAS group, there were 9 hypersensitivity reactions, including one death from acute yellow atrophy. The great bulk of PAS toxicity was associated with gastrointestinal upset.

Sumario

Comparación de la Cicloserina–Isoniacida con la Isoniacida–Acido Para-Aminosalicílico en la Terapéutica de la Tuberculosis Pulmonar Cavitaria: X. Comunicación del Estudio Cooperativo de la Administración de Veteranos y las Fuerzas Armadas

Se analizan los datos derivados del estudio cooperativo de la Administración de Veteranos y las Fuerzas Armadas acerca de la eficacia clínica de las dos drogas cicloserina e isoniacida usadas juntas. Se comparó la eficacia de este régimen con la de isoniacida y el ácido para-aminosalicílico (PAS), administrados concurrentemente a un grupo testigo de enfermos que tenían tuberculosis cavitaria en catorce hospitales que cooperaron en el estudio.

En varios sentidos, los resultados obtenidos en el grupo tratado con cicloserina–isoniacida tendieron a demostrar que este régimen es menos eficaz que el de isoniacida–PAS. Estos resultados son: una incidencia mayor de signos radiográficos de avance de la enfermedad, 7 por ciento, comparado con una incidencia de 2 por ciento en el grupo testigo; una incidencia mucho mayor de fracasos clínicos en la falta respuesta satisfactoria al tratamiento, lo cual exigió un cambio del régimen medicamentoso en 12 por ciento de los casos, comparado con 2 por ciento en el grupo tratado con isoniacida–PAS; una incidencia menor de cierre de cavernas; una incidencia menor de aparición de negatividad bacteriológica y de "viraje" bacteriológico de las secreciones broncopulmonares; aparición más rápida de resistencia bacteriana a la isoniacida con una incidencia global mayor de resistencia a la isoniacida.

Aunque (en general) bien tolerado, no carece el régimen de su propia toxicidad, según patentizan la aparición de un acceso convulsivo, un caso de neuritis óptica, 3 reacciones de hipersensibilidad y quizás 13 casos de aberración mental o alteraciones del comportamiento. En el grupo de isoniacida–PAS, hubo 9 reacciones de hipersensibilidad, comprendiendo una muerte de atrofia amarilla aguda. La mayor parte de la toxicidad del PAS se asoció con perturbaciones gástricas.

Resume

Comparaison entre l association cyclosérine–Isoniazide et isoniazide–acide para-aminosalicylique dans le traitement de la tuberculose pulmonaire avec cavernes. X. Rapport d une étude comparée menée par l'Administration des Vétérans et les Forces Armées

L'auteur analyse les renseignements fournis par une étude faite par l'Administration des Vétérans en assoçiation avec les Forces Armées,

sur l'efficacité clinique de ces deux médicaments, cyclosérine et isoniazide utilisés ensemble. L'efficacité de ce traitement fut comparé à celle de l'isoniazide et de l'acide para-aminosalicylique (PAS) donnés en même temps à un groupe de contrôle de malades porteurs de cavernes tuberculeuses, dans quatorze hopitaux affiliés.

Pour plusieurs raisons, les résultats du groupe traité par la cyclosérine et l'isoniazide semblent montrer que ce traitement est moins efficace que l'assoçiation isoniazide–PAS. Ces résultats sont: une fréquence plus grande de la preuve radiographique de la progression de la maladie, 7% en comparaison d'une fréquence de 2% parmi le groupe de contrôle; une fréquence beaucoup plus grande d'échec clinique comme réponse au traitement, ce qui nécessita de changer le traitement chimique dans 12% des cas, en comparaison de 2% dans le groupe traité par l'isoniazide–PAS; une fréquence moins grande de fermeture de la caverne; une fréquence de cultures bactériologiques négatives et de "conversion" bactériologique des secrétions bronchopulmonaires; l'apparition plus rapide d'organismes résistant à l'isoniazide avec une fréquence générale plus grande de résistance à l'isoniazide.

Bien qu'en général bien toléré, le traitement n'est pas sans sa propre toxicité, ainsi que le montre l'apparition d'une crise convulsive, un cas de névrite optique, trois réactions d'hypersensibilité et peut-être treize cas de troubles mentaux ou de changement de comportement. Dans le groupe isoniazide–PAS, il y eut neuf réactions d'hypersensibilité, y compris un cas de mort par atrophie jaune aigüe. Dans la plupart des cas de troubles dus à la toxicité du PAS, il y eut assoçiés des troubles gastro-intestinaux.

Acknowledgments

Data for this cooperative study were contributed by the staffs of the following Veterans Administration hospitals: Albuquerque, New Mexico; Baltimore, Maryland; Durham, North Carolina; East Orange, New Jersey; Houston, Texas; Indianapolis, Indiana; Livermore, California; Madison, Wisconsin; Martinsburg, West Virginia; Memphis, Tennessee; Minneapolis, Minnesota; Oteen, North Carolina; Richmond, Virginia; San Fernando, California; Sunmount, New York; and Tucson, Arizona.

The data submitted by these participating hospitals were compiled by the Medical Research Statistics Division of the Veterans Administration Central Office, Washington, D. C. under the direction of Mrs. Dorothy G. Livings.

The writer could not close without due acknowledgement of the careful scrutiny, thoughtful criticism, and advice rendered in the many preparations of this material by Doctors William B. Tucker, Edward Dunner, James W. Raleigh, Clifford A. Bachrach, and the members of the Executive Committee of the Veterans Administration–Armed Forces Cooperative Study Group; nor without expressing appreciation to Mrs. Julia M. Iman and Mrs. Carolyn A. Bevans for their careful secretarial work on the several manuscripts prepared in the course of the study, and to Mr. Rowland B. Schnick, who is Chief of the Medical Illustration Service, at the Baltimore Hospital.

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THE SUPPRESSED MULTIPLICATION OF TUBERCLE BACILLI WITHIN MACROPHAGES DERIVED FROM TRIIODOTHYRONINE-TREATED GUINEA PIGS[1]

H. S. HSU AND FRANK A. KAPRAL

(Received for publication September 14, 1959)

INTRODUCTION

Recent observations have shown that hyperthyroidism, as induced by triiodothyronine or thyroxine, markedly enhanced the resistance of certain strains of inbred rabbits to tuberculosis (1, 2). Lurie hypothesized that hyperthyroidism altered the physiologic activity of the phagocytes and thus enhanced the suppression of intracellular multiplication of tubercle bacilli.

The essential immune mechanism involved in "reinfection" tuberculosis has long been attributed by some workers to the increased bacteriostatic capacity of the macrophages. Lurie (3) introduced normal and "immune" rabbit mononuclear macrophages infected with tubercle bacilli separately into the opposite anterior chambers of the eyes of normal rabbits. He demonstrated by cultural methods that, after two weeks of growth in an apparently identical *in vivo* environment, the tubercle bacilli in the "immune" cells exhibited a slower rate of multiplication as compared with that in the normal cells. Suter (4) and Berthrong and Hamilton (5) have also observed that, by maintaining monocytes infected with tubercle bacilli in tissue culture, the intracellular multiplication of these microorganisms was suppressed by cells from immunized guinea pigs or rabbits in contrast to the multiplication observed in monocytes from normal animals.

Mackaness (6), on the contrary, failed to substantiate Suter's findings and observed no basic difference in the intracellular multiplication of tubercle bacilli in the cells of immunized or normal rabbits. Fong, Schneider, and Elberg (7) reported that the presence of "immune" serum exerted a favorable effect upon survival of normal monocytes infected with tubercle bacilli and a complete inhibition of degeneration of infected "immune" cells. Apparently conflicting observations by these workers may be due to their different working criteria used in the tissue culture method. Suter, Mackaness, and Berthrong and Hamilton followed the intracellular increase of tubercle bacilli in stained preparations, whereas Fong, Schneider, and Elberg based their end point on the visible "degenerative" effect of tubercle bacilli on monocytes.

While the tissue culture technique undoubtedly offers an opportunity to study host responses on a cellular level and to explore the relative roles of humoral and cellular factors, a critical evaluation of data presents a challenge. Since both heat-killed and streptomycin-killed tubercle bacilli remain acid fast in stained preparations, and since both the accuracy of microscopic counting of intracellular bacilli and the "degenerative" effects on monocytes by tubercle bacilli appear questionable, a precise approach to such studies calls for a quantitative recovery of viable intracellular parasites at different intervals of observation.

The purpose of the present study was, in part, to test the validity of Lurie's hypothesis by observing the intracellular multiplication of tubercle bacilli in monocytes derived from triiodothyronine-treated and untreated guinea pigs. A technique for the quantitative recovery of intracellular tubercle bacilli was devised to follow the rate of increase of the intracellular bacterial population.

MATERIALS AND METHODS

Mycobacterium tuberculosis: Two strains of H37Rv were used in this study.[2] One strain (H37Rv-P),[3] maintained for many years in Dr. Lurie's laboratory, was somewhat attenuated, whereas a newly obtained strain (H37Rv-S)[3] was more tuberculogenic (8). Cultures of these organ-

[1] From the Department of Microbiology, School of Medicine, University of Pennsylvania, Philadelphia 4, Pennsylvania.

[2] Supplied through the kindness of Dr. Max B. Lurie, Henry Phipps Institute, Philadelphia, Pennsylvania.

[3] Strain H37Rv-P was previously designated by Dr. Max B. Lurie as H37Rv-Phipps, and strain H37Rv-S as H37Rv-Steenken (*cf.* reference 8).

isms were maintained in Dubos Tween®-80 medium and transplanted weekly. Screw-capped tubes containing approximately 7 ml. of the liquid medium were used, and cultures were incubated in a roller tube apparatus rotating at 9 r.p.m. at 37°C. To obtain an isolated bacterial suspension for parasitization of monocytes, a seven- to nine-day-old culture was centrifuged at 200 × g. for five minutes and the supernate was transferred into another tube and recentrifuged at 500 × g. for five minutes. The supernate contained chiefly single bacteria with a few small clumps of no more than 3 to 4 bacteria each.

Animals: Albino male guinea pigs were purchased from commercial sources and were kept on diets of guinea-pig pellets and greens. In view of the pharmacologic effects of triiodothyronine, animals weighing more than 700 gm. were found better able to survive treatment with this hormone. Before being used for an experiment, each guinea pig was checked for any evidence of streptococcal lymphadenitis, and only apparently healthy animals were used in this study. This was done to avoid encountering a cross resistance which might perhaps be induced by this organism.

Treatment of guinea pigs: Hyperthyroidism in guinea pigs was induced by the daily injections of 10 γ of L-3:5:3′-triiodothyronine[4] over a period of approximately two weeks. The state of hyperthyroidism was confirmed by an increase of basal metabolic rate toward the end of treatment. The basic metabolic rate of normal control animals was also determined over the same period as that used with the treated animals.

Cultivation of monocytes infected with tubercle bacilli: Exudates were induced by the intraperitoneal injection of 10 ml. of mineral oil[5] two or three days prior to harvesting the cells. The animals were killed with a blow on the head. The peritoneal cavity was aseptically washed with 20 to 25 ml. of Hanks solution containing 2 per cent normal guinea pig serum and heparin[6] 1:10,000. The pH was adjusted to 7.4 with isotonic bicarbonate solution.

The Hanks solution was introduced as small aliquots. The peritoneum was carefully washed and the fluid withdrawn with a bulb pipette after adding each aliquot of Hanks solution. The washings were placed in test tubes and permitted to stand for a few minutes so that the mineral oil rose to the top. The leukocyte suspension was then transferred to a clean test tube and its pH was immediately adjusted to 7.2. The usual leukocyte suspension consisted of approximately 10 to 12 ml., with a total cell count of 4 to 5 million leukocytes per ml., more than 50 per cent being monocytes.

The bacterial suspension in Dubos medium, obtained as described above, was mixed with the leukocyte suspension in a test tube in a volume ratio varying between 1:5 and 1:7, respectively. The mixture was placed for thirty to forty minutes in a roller tube apparatus rotating at 26 r.p.m. to permit phagocytosis of the bacteria.

In the meantime, tissue culture flasks were prepared for the reception of the infected leukocytes. Porter flasks containing four coverslips of equal size (5 by 18 mm.) were sterilized in the hot-air oven. Approximately 0.3 ml. of Hanks solution was pipetted into each flask so that the coverslips were thoroughly wetted and could be aligned parallel to each other on the bottom of the flask. The coverslips were easily manipulated with a platinum wire bent to have a small hook at the end.

After phagocytosis, an estimated population of 5 million leukocytes was introduced into each Porter flask and left in the incubator (37°C.) for twenty minutes to allow the cells to settle onto the coverslips. The excess fluid was then poured from each flask and the adhering cells were washed once with 1.5 ml. of Hanks solution and reincubated with 1.5 ml. fresh Hanks solution for ten minutes.

After the second incubation period, the Hanks solution in each flask was replaced with 1.5 ml. of tissue culture medium. The tissue culture medium consisted of 20 per cent pooled normal guinea pig serum, 65 per cent Hanks solution, and 15 per cent isotonic 1.4 per cent sodium bicarbonate solution (freshly saturated with carbon dioxide just before use). Concentrations of 30 units of nystatin, 50 units of penicillin, and 5 γ of streptomycin per ml. were used in the medium to prevent contamination and extracellular multiplication of tubercle bacilli.

Since polymorphonuclear leukocytes rarely survived overnight, such cultures contained almost 100 per cent monocytes. The tissue cultures were then incubated for study over a period up to twelve days. The medium was maintained at pH 7.2–7.4 throughout the experiment and, on the third day, 1 ml. of medium from each flask was removed and replaced with fresh medium.

Six flasks were usually prepared from cells of each animal. Two coverslips were removed from each of the duplicate flasks at each designated interval. One coverslip was stained in order to enumerate the cell population; the other was used to determine the culturable intracellular tubercle bacilli.

Recovery of intracellular tubercle bacilli and evaluation of data: Five-tenths of 1 per cent solution of sodium desoxycholate was found to cause an immediate disintegration of leukocytes upon contact, yet had no effect on the viability of tubercle bacilli even after a contact of ten minutes. This concentration of sodium desoxycholate was, therefore, used as a means of disrupting the monocytes in order to recover the intracellular tubercle bacilli.

At various intervals, two coverslips were removed from each of the duplicate flasks and washed in Hanks solution containing 10 per cent

[4] Manufactured as Cytomel Sodium, supplied through the courtesy of Smith, Kline and French Laboratories of Philadelphia, Pennsylvania.

[5] Parke Davis & Co., heavy or light mineral oil.

[6] Liquaemin Sodium, Organon, Inc.

normal guinea pig serum. One of the coverslips was fixed in methanol and later stained by the Ziehl-Neelsen method. The other coverslip was placed in 1 ml. of 0.5 per cent sodium desoxycholate solution in a Wassermann tube. The contents were thoroughly mixed by pipetting forty to fifty times with a 1-ml. pipette to ensure complete disintegration of monocytes and uniform resuspension of the released tubercle bacilli. A 0.3 ml. aliquot of this suspension was transferred to a test tube containing 5.7 ml. of sterile saline (1:20 dilution) and thoroughly mixed with a 10-ml. pipette. Using a fresh pipette, the suspension was diluted another tenfold and two 0.5 ml. aliquots of this final dilution were plated out by the pour-plate method. Colony counts were made after a twenty- to twenty-three-day incubation period at 37°C. Dubos Tween-80 medium solidified with 2 per cent Difco's Noble Agar was used for all plate counts in this study.

From the plate counts, the total number of culturable intracellular tubercle bacilli present on each coverslip could be calculated. From the stained coverslip, the total cell population per coverslip was estimated by counting the number of cells in a total of forty to fifty microscopic fields. Since the distribution of cells over the bottom of a Porter flask tended to be fairly uniform, the leukocyte population on each coverslip within the same flask would be equivalent. By dividing the total number of tubercle bacilli released from within the monocytes by the total number of cells present, the average number of bacilli per monocyte could be determined at each sampling time.

Results

Triiodothyronine treatment of guinea pigs: For each experiment, 2 guinea pigs were used, one having been treated with 10 γ of triiodothyronine daily over a period of approximately two weeks; the other being used as an untreated control. In some instances 20 γ of triiodothyronine were given daily two or three days prior to the date of experiment instead of the usual dosage of 10 γ. Such hormone treatment normally imposed a 10 to 15 per cent loss in body weight of the animal, and conferred a basal metabolic rate increase of 20 to 40 per cent, while the basal metabolic rate of untreated controls varied between plus and minus 10 per cent.

Intracellular multiplication of H37Rv-P: Monocytes from triiodothyronine-treated and untreated guinea pigs were infected with the H37Rv-P strain and maintained in tissue culture for a period up to ten days. The intracellular tubercle bacilli were recovered and enumerated at different sampling times. The ratio of the number of tubercle bacilli per cell found at each sampling and the number of tubercle bacilli originally phagocytized per cell are plotted in figure 1.

The data illustrate that this strain of H37Rv survived in the monocytes for a period of four to five days. This lag phase was followed by an approximately fourfold increase in the intracellular bacilli over the next two to three days. This growth phase appeared somewhat suppressed in the cells from the treated animals. After seven days, there did not seem to be any further significant increase in bacterial numbers, irrespective of the source of the macrophages.

Intracellular multiplication of H37Rv-S: Monocytes of both treated and normal guinea pigs were infected with the H37Rv-S strain, and the intracellular multiplication was compared as previously described (figure 2). It is evident from the data that the population of tubercle bacilli within the normal monocytes doubled in about two days after phagocytosis, whereas there was little or no growth of the comparable population in the monocytes from the treated animals. This strain of bacteria demonstrated its ability to multiply in the normal monocytes continuously throughout the period of nine days, with a generation time of two to three days, while the generation time of the same strain in monocytes of treated animals was five days or more.

The possibility existed that cells from triiodothyronine-treated animals were more susceptible to rupture by the increase of intracellular tubercle bacilli and, if this were the case, then the present writers' conclusions might be invalidated. That such an increased susceptibility did not occur was demonstrated in the following manner. In one experiment from each of the two strains of the H37Rv reported here, 200 cells were counted from each stained preparation according to the procedure described by Suter (9). The number of intracellular organisms was grouped in the following columns: 0, 1 to 5, 6 to 10, 11 to 15, and more than 16. In general, it was observed that approximately 20 per cent of the cell population was initially infected with tubercle bacilli. But this value shifted so that, at the end of the experimental period, about 80 per cent of the surviving cell population contained tubercle bacilli. It should be borne in mind that, in such a dynamic system of intracellular multiplication, bacteria were continuously released into the medium by ruptured leukocytes. Although the tubercle bacilli were presumably killed by the

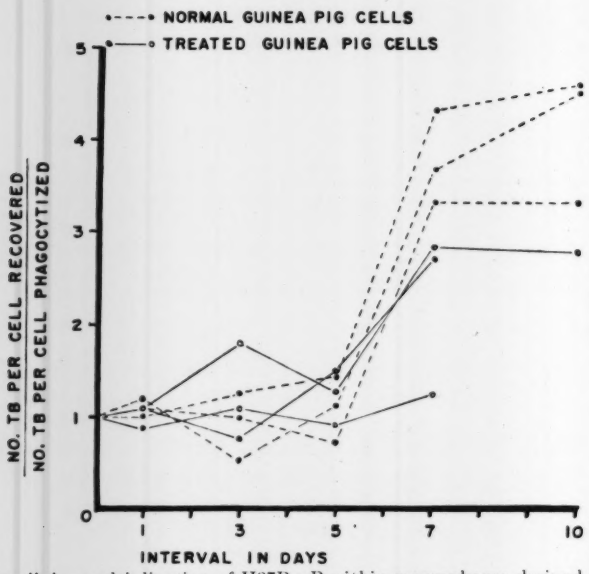

FIG. 1. The intracellular multiplication of H37Rv-P within macrophages derived from triiodothyronine-treated and untreated guinea pigs.

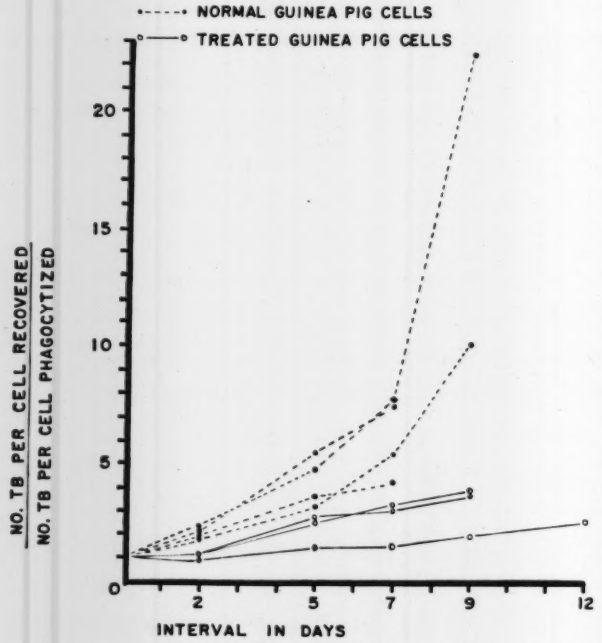

FIG. 2. The intracellular multiplication of H37Rv-S within macrophages derived from triiodothyronine-treated and untreated guinea pigs.

streptomycin in the medium, the organisms still remained acid fast and could be rephagocytized by the surviving cells. Hence this would make interpretation of the data by this technique difficult. In the experiment in which the H37Rv-P strain of tubercle bacilli was used, 25 per cent of the cells from the untreated animal and 4 per cent of the cells from the treated animal concontained 16 or more organisms on the tenth day. In the experiment using the H37Rv-S strain, 12 per cent of the cells from the untreated animal contained more than 16 bacteria per cell on the seventh day, while only 3 per cent of the comparable cells from the treated animal were in the same category at that time. By the twelfth day, however, the proportion of cells from the treated animal containing at least 16 organisms had increased to 10 per cent. Therefore, cells from treated animals were just as capable of containing large numbers of tubercle bacilli as were cells from untreated animals.

The results indicated that the two strains of H37Rv used in this study do behave differently with respect to their abilities to multiply in macrophages from normal guinea pigs. This is analogous to the observation of Lurie and associates (8) concerning the difference in ability of these two strains of H37Rv to generate tubercles in rabbits.

Discussion

The technique employed in this study provides a precise and quantitative means of following the intracellular multiplication of tubercle bacilli. This procedure determines the actual number of culturable intracellular parasites. Recovery of intracellular bacteria *in vitro* has previously been attempted, though not quantitatively, by Pomales-Lebron and Stinebring (10) in their demonstration of cellular immunity in brucellosis. While the present investigation was in progress, Elberg, Schneider, and Fong (11) and Holland and Pickett (12) have separately published their observations on cellular immunity in brucellosis by cultural methods; and Kapral and Shayegani (13) reported on the fate of intracellular staphylococci, using a technique similar to that reported here.

The effects of hyper- and hypothyroidism on clinical tuberculosis have long been noted. The earlier experimental effort to confirm these was made by Izzo and Cicardo (14) in their report that injections of thyroxine increased, whereas thyroidectomy decreased, the resistance to experimental tuberculosis in guinea pigs. These studies were based on differences in survival time after infection under these conditions. These effects were recently studied in greater detail by Lurie and his associates (2, 8). The beneficial effects of hyperthyroidism cannot be generalized with respect to other animal species. In fact, thyroid hormone treatments were found by Dubos (15) and Nutter and co-workers (16) to have an adverse result on tuberculosis in mice. The practical application of triiodothyronine treatment, furthermore, has demonstrated no apparent increased resistance in tuberculous humans as was reported by Nodine and associates (17). In the last-named case, however, the effects of hormone treatment may have been masked by the simultaneous antituberculous therapy and, furthermore, the patients were only mildly thyrotoxic in contrast to the severe hyperthyroidism suffered by experimental animals. The present study attempts to follow the fate of tubercle bacilli in macrophages of hyperthyroid guinea pigs.

It is shown in figure 2 that the H37Rv-S strain began to multiply within two days after phagocytosis by normal macrophages, whereas there was little multiplication in the H37Rv-P strain before five days (figure 1). This is in agreement with the observation of Lurie and associates that the H37Rv-P strain is somewhat attenuated and less virulent than the H37Rv-S strain (8) if virulence is defined as the ability of the bacteria to establish themselves and to multiply in the host.

In a culture of uninfected monocytes, the cell population declined rapidly within the first three days and slowly leveled off. Monocytes did not multiply. Microscopic studies of the stained coverslips in the experiments reported here showed that occasional cells might contain two nuclei, possibly as a result of fusion rather than amitosis, but in no case were mitotic figures observed. Furthermore, the infected cell population generally declined faster in the cells of the untreated animal as compared to those of the treated one. This could be explained by the higher rate of intracellular multiplication of bacteria in the former case, which in turn ruptured the leukocytes more rapidly. That cells from triiodothyronine-treated animals were not more susceptible to rupture by intracellular tubercle bacilli was shown by the ability of these

cells to contain just as many organisms as cells from normal animals.

It should be emphasized that the data illustrated in this study were presented as the average number of tubercle bacilli per cell in the surviving population and that the rate of bacterial increase was based on the comparison of this ratio at a specific time with that found at the zero time. Thus, the change in cell population with time had no bearing on this evaluation.

The 5 γ of streptomycin per ml. in the tissue culture medium were necessary to inhibit extracellular growth of tubercle bacilli. Preferential permeability of streptomycin into cells from treated animals remains a possibility. To test this possibility experimentally would not be easy. However, as the concentration of this antimicrobial was small and there was actual multiplication of tubercle bacilli in cells from treated animals, the chance that the suppression of intracellular multiplication by these cells was merely due to a preferential permeability of streptomycin seems remote.

Over the past decade, much interest in host–parasite relationships has been focused at a cellular level. Mounting evidence has pointed to the role of cellular immunity as the most important immune mechanism in tuberculosis and brucellosis. It is conceivable that cellular environments could exist that would be less favorable for the growth of the parasite and that the occurrence of such an environment might be the result of a previous infection, specific drug action, or even genetic influence. This alteration in environment would be reflected in the biochemistry of the cell. The studies reported here demonstrate such a change, in which the capacity for suppressing intracellular multiplication of tubercle bacilli was induced in the host by the administration of a single hormone. It would be of interest, in future studies, to follow host cell and parasite interactions with this concept, namely, a change in the physiology of the host may impose a different growth behavior on the intracellular parasite.

Summary

A technique is presented which permits the intracellular multiplication of human tubercle bacilli *in vitro* to be followed in a quantitative manner.

The intracellular growth characteristics of the two strains of H37Rv tubercle bacilli used in this study were shown to be different. The H37Rv-P strain exhibited a lag phase of four to five days before multiplication occurred. There was a two- to three-day growth phase, after which the intracellular population remained essentially constant. In monocytes derived from triiodothyronine-treated guinea pigs, the intracellular multiplication of the parasite appeared slightly suppressed. The H37Rv-S strain, on the other hand, had the ability to multiply within normal monocytes at an almost constant rate throughout a nine-day period, with a generation time of two to three days. There was no appreciable lag phase. Multiplication of this strain in monocytes from treated animals was retarded, having a lag phase of about two days and a subsequent generation time of approximately five days.

This study demonstrates the presence of "cellular resistance" which was induced in guinea pigs by a naturally occurring entity without previous infection or vaccination, and in which humoral factors did not appear to play a prominent role.

Sumario

La Multiplicación Suprimida de los Bacilos Tuberculosos dentro de Macrófagos Derivados de Cobayos Tratados con Triyodotironina

Preséntase una técnica que permite observar en forma cuantitativa la multiplicación intracelular de los bacilos tuberculosos humanos *in vitro*.

Se demostró que eran distintas las características de la proliferación intracelular de las dos cepas de bacilos tuberculosos H37Rv usadas en este estudio. La cepa H37Rv-P manifestó una fase de retardo de cuatro a cinco días antes de haber multiplicación. Hubo una fase de crecimiento de dos a tres días, después de la cual la población intracelular permaneció más o menos constante. En los monocitos derivados de los cobayos tratados con triyodotironina, la multiplicación intracelular del parásito pareció estar ligeramente suprimida. En cambio, la cepa H37Rv-S desplegó capacidad para multiplicarse dentro de los monocitos normales a una velocidad casi constante durante un período de nueve días, con un tiempo de generación de dos a tres días. No hubo apreciable fase de retardo. En cambio, se retardó sí la multiplicación de esta cepa en los monocitos procedentes de animales tratados, mostrando una fase de retardo de unos dos días y un tiempo subsiguiente de generación de unos cinco días.

Demuestra este estudio la existencia de "resistencia celular," que se indujo en los cobayos con una entidad existente en la naturaleza, sin

previa infección o vacunación, y en la cual los factores humorales no parecen desempeñar un papel destacado.

Resume

Suppression de la multiplication des bacilles tuberculeux dans les macrophages provenant de cobayes traités par la triiodothyronine

Les auteurs présentent une technique que permet de suivre d'une manière quantitative la multiplication intracellulaire de bacilles tuberculeux *in vitro*.

Il apparut que les caractéristiques de développement intracellulaire de deux souches de bacilles tuberculeux H37Rv utilisées dans cette étude sont différentes. La souche H37Rv-P montra une phase d'incubation de quatre ou cinq jours avant que la multiplication ne se produisit. Il y eut une phase de développement de deux ou trois jours après laquelle la population intracellulaire resta essentiellement constante. Dans les monocytes issus de cobayes traités par la triiodothyronine la multiplication intracellulaire du parasite fut légèrement enrayée. D'un autre coté, la souche H37Rv-S se montra capable de se multiplier dans des monocytes normaux à un rythme presque constant pendant une période de neuf jours, avec une durée de génération de deux à trois jours. Il n'y eut pas de phase d'incubation appréciable. La multiplication de cette souche dans les monocytes de cobayes traités fut retardée, ayant une phase d'incubation d'à peu près deux jours et une durée de génération d'à peu près cinq jours.

Cette étude démontre la présence d'une "résistance cellulaire" qui fut suscitée chez les cobayes d'une façon naturelle, sans infection ou vaccination préalable, et pour laquelle les facteurs humoraux ne semblèrent pas jouer un rôle important.

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UNCLASSIFIED MYCOBACTERIA IN THE GASTRIC CONTENTS OF HEALTHY PERSONNEL AND OF PATIENTS OF A TUBERCULOSIS HOSPITAL[1, 2]

ROBERT J. ATWELL AND PHILIP C. PRATT

(Received for publication September 9, 1959)

INTRODUCTION

The presence of unclassified mycobacteria[3] in man poses a real problem in the diagnosis of pulmonary disease. Undoubtedly some of these organisms can produce disease, but their true and complete significance is still largely unknown. Runyon (1) has recently reviewed this subject in detail.

The present study was undertaken in an attempt to investigate the significance of unclassified mycobacteria in patients and to compare the incidence of these organisms in patients and in normal healthy persons. For purposes of simplicity, the name unclassified mycobacteria will be used to refer to the whole group of acid-fast organisms other than *M. tuberculosis*. Unless specifically stated otherwise, "positive culture" refers to unclassified mycobacteria which will be further described according to the scheme of Runyon (1).

MATERIALS AND METHODS

One hundred and twelve employees of the Ohio Tuberculosis Hospital volunteered for the study of gastric contents. These employees were of known skin reactivity and all have had roentgenograms of the chest every three months throughout their employment, during the period of the study, and subsequently. All were free of any clinical or roentgenographic evidence of pulmonary disease except for occasional scattered calcifications, and were free of any history of pulmonary disease in the past.

The first period of study covered March to July, 1957. The employees were instructed to refrain from eating or drinking after the evening meal and to report to the hospital between six and eight a.m., as soon after arising as possible. Single gastric aspirations were obtained by means of a Levin tube. In this procedure the tube was cooled by packing in ice and was lubricated with sterile mineral oil. The gastric contents were obtained by passing the tube through the nose and into the stomach. Gastric contents were then concentrated by treating with 4 per cent sodium hydroxide followed by agitation and then centrifugation. The sediment was neutralized with 1 N. HCl, then smeared, stained with Ziehl-Neelsen stain, and examined for acid-fast bacilli. The remainder of the sediment was cultured on two tubes of Löwenstein medium. These cultures were incubated for eight weeks in total darkness at 37°C. and inspected weekly for signs of growth. Any growth which appeared was described as to color and colony morphology, then smeared, and stained for acid-fastness. All acid-fast organisms were tested for susceptibility to isoniazid, streptomycin, and PAS, and for virulence in guinea pigs.

During the same period in 1957 the gastric aspirates of 30 consecutive patients were likewise studied for comparison with the results in personnel. The technique of gastric aspiration and culture was exactly the same as that used in the employee group.

One year later, during the first six months of 1958, 87 of the original volunteer employees each had two gastric aspirations. In this series, sterile lubricant jelly was substituted for the sterile mineral oil and the gastric tubes were cooled in a freezer without unwrapping the sterile pack. Smears and cultures of the gastric sediment, guinea pig inoculation, and susceptibility studies were done as in the earlier study. Nasal smears were also prepared and stained for acid-fast organisms.

During the first ten months of 1958, 127 patients with gastric aspirations were compared with the employees. Forty of these patients from January to May, 1958, had gastric aspirations utilizing sterile mineral oil and icing of the tubes; 87 patients from May to October, 1958, had gastric aspirations using sterile lubricant jelly and tubes chilled in a freezer rather than by icing. All of the reagents used in preparation of the gastric contents were sterilized daily by autoclaving before use.

RESULTS

The results of the first series of gastric aspirates in employees and patients are presented in table 1. In the employee group, 21 cultures were

[1] From the Ohio Tuberculosis Hospital, and the Department of Medicine, The Ohio State University, Columbus, Ohio.

[2] This work was supported by a grant from the Ohio Tuberculosis and Health Association. Statistical analysis was carried out by the Statistical Analysis Unit, Division of Vital Statistics, Ohio Department of Health.

[3] Unclassified mycobacteria is the terminology adopted by the REVIEW for such terms as *atypical* or *anonymous* acid-fast bacilli.

positive for unclassified mycobacteria, 18 of which were orange scotochromogens and 3, smooth, buff-colored nonphotochromogens. One culture was positive for *M. tuberculosis*. All of these organisms grew slowly. The culture of *M. tuberculosis* produced fatal disease in a guinea pig and was susceptible to all drugs. Repeated gastric aspirations and close follow-up study of the roentgenogram of this employee failed to reveal any evidence of tuberculosis, nor was another positive gastric culture obtained.

The 10 cultures of unclassified mycobacteria found in the patient group were all of the orange, scotochromogen type, and all were slow growing.

TABLE 1

UNCLASSIFIED MYCOBACTERIA IN GASTRIC CONTENTS OF EMPLOYEES AND PATIENTS, MARCH–JULY, 1957

Old Technique

	Employees	Patients
Total persons	112	30
Total gastric washings	112	101
Number of positive smears	2	
Number of positive cultures*	21	10
Per cent of positive cultures	18.8	9.9
Number of persons with positive cultures	21	8
Per cent of persons with positive cultures	18.8	26.7

* "Positive" signifies the presence of unclassified mycobacteria only.

All but one of the patients were known to have tuberculosis, and it was believed that the pulmonary disease in that case was not due to unclassified mycobacteria. The other 7 all had bacteriologically proved tuberculosis, but the sputum of most had become negative for tubercle bacilli prior to the study. One patient had one culture positive for both *M. tuberculosis* and an unclassified mycobacteria. All had reactive tuberculin skin tests.

Organisms obtained from the employees were all catalase positive and resistant to isoniazid, PAS, and streptomycin except for the one culture of *M. tuberculosis*. No evidence of disease was produced in guinea pigs by the unclassified mycobacteria except for a weakly reactive tuberculin skin test.

The results of the gastric aspirations during the second period of study are presented in table 2. During this study, all cultures from both personnel and patients were orange scotochromogens of the slow-growing variety, and none of the organisms from the employees produced disease in guinea pigs except for weakly reactive tuberculin skin tests. The organisms obtained from the employees were all catalase positive and resistant to the three major antituberculous drugs.

No employee had more than one culture positive for unclassified mycobacteria during both periods of study, whereas several patients did have two such cultures. Of the 21 employees from whom unclassified mycobacteria were ob

TABLE 2

UNCLASSIFIED MYCOBACTERIA IN GASTRIC CONTENTS OF EMPLOYEES AND PATIENTS, JANUARY–OCTOBER, 1958

Effect of Technique Changes

	Employees	Patients	
	Improved Technique January–July, 1958	Old Technique January–May, 1958	Improved Technique May–October, 1958
Total persons	87	40	87
Total gastric washings	173	184	296
Number of positive smears	9		
Number of positive cultures*	4	17	8
Per cent of positive cultures	2.3	9.2	2.7
Number of persons with positive cultures	4	11	8
Per cent of persons with positive cultures	4.6	27.5	9.2

* "Positive" signifies the presence of unclassified myocbacteria only.

tained in the first series, 15 were restudied, with negative findings in all.

Of the 19 patients yielding cultures positive for unclassified mycobacteria in the second series, 3 had cultures positive for *M. tuberculosis* during the same period, 5 did not have tuberculosis, and none were thought to have disease caused by the unclassified mycobacteria. Three of the 5 nontuberculous patients had nonreactive tuberculin skin tests.

TABLE 3

INCIDENCE OF UNCLASSIFIED MYCOBACTERIA IN SPUTUM AND GASTRIC CONTENTS OF PATIENTS WITH PULMONARY DISEASE, SEPTEMBER, 1958–APRIL, 1959

Source	Sputum			Gastric Contents		
Diagnosis	Tuberculosis	Nontuberculous Disease	Unknown	Tuberculosis	Nontuberculous Disease	Unknown
Total patients	229	28	5	86	12	4
Total cultures	1,776	101	21	361	38	13
Positive cultures*	10	0	1	9	2	1
Per cent of positive cultures	0.6			2.5		
Patients with positive cultures	9	0	1	8	2	1
Per cent of patients with positive cultures	3.9			9.4		

* "Positive" signifies the presence of unclassified mycobacteria only.

All nasal smears of the employees were negative for acid-fast bacilli. Attempts to culture mycobacteria from ice, tap water, and mineral oil were not successful.

The incidence of unclassified mycobacteria in sputum cultures as compared with gastric cultures was also examined. For this purpose 5 patients who had sputum cultures consistently positive for unclassified mycobacteria and negative for *M. tuberculosis* were excluded because they were considered to have disease caused by unclassified mycobacteria. The data on the comparative incidence of unclassified mycobacteria in sputum and gastric culture in patients are presented in table 3. All of the unclassified mycobacteria were scotochromogens. None of the 8 patients who had both sputum and gastric cultures had cultures positive for unclassified mycobacteria on both types of examination. Of the 7 patients with gastric cultures positive for unclassified mycobacteria, 8 of 32 cultures were positive and 33 sputum cultures were negative. One patient with 1 of 20 sputum cultures positive for mycobacteria had 6 negative gastric cultures.

DISCUSSION

The data have been subjected to statistical analysis. Associated with the changes in technique of gastric aspiration, there was a significant reduction in the incidence of unclassified mycobacteria in employees (18.8 per cent before change, 2.3 per cent after) and in patients (9.2 per cent before change, 2.7 per cent after). There was no significant difference in the incidence of unclassified mycobacteria between employees (2.3 per cent) and patients (2.7 per cent) when the same technique of aspiration was used. There was no

TABLE 4

ADJUSTED DATA COMPARING THE FIRST TWO GASTRIC CULTURES IN PATIENTS AND PERSONNEL
Collection Technique Improved

Item	First Test		Second Test		First and Second Tests	
	Employees	Patients	Employees	Patients	Employees	Patients
Total persons	87	87	86	87	87	87
Total gastric washings	87	87	86	87	173	174
Number of positive cultures*	2	5	2	0	4	5
Per cent of positive cultures	2.3	5.7	2.3	0.0	2.3	2.9
Number of persons with positive cultures	2	5	2	0	4	5
Per cent of persons with positive cultures	2.3	5.7	2.3	0.0	4.6	5.7

* "Positive" signifies the presence of unclassified mycobacteria only.

difference in the incidence of unclassified mycobacteria by three-month intervals from January through October.

The difference in the percentage of persons positive seems rather large at first glance. These data were subjected to further analysis, using only the first two gastric washings obtained in each patient compared with the results in the employees. These adjusted data are presented in table 4. There was no significant difference in the incidence of unclassified mycobacteria comparing the first cultures, the second cultures, or the first and second cultures of employees (4.6 per cent) and patients (5.7 per cent). This suggests that the apparent difference in the total data (table 2) is a result of the greater number of specimens cultured in the patient group.

The incidence of unclassified mycobacteria in sputum cultures of patients with pulmonary disease has been stated to be from 0.5 to 4 per cent, with a patient incidence of 1.7 to 32 per cent (2–9). The incidence in gastric contents has been stated to be higher (3), ranging from 0 to 25 per cent (4, 9, 10) in nontuberculous patients and from 0 to 1.8 per cent in healthy persons (6, 11). The incidence in patients with tuberculosis has been reported as high as 32 per cent (12). Most of these unclassified mycobacteria are scotochromogens which rarely produce disease (1).

The marked variation in incidence has no proved explanation at this time. Acid-fast bacilli have been described in various reagents, in tap water, in various foods, in cold-blooded animals, as saprophytes in man, in grasses, and almost anywhere in nature (13–15). Since these organisms are so common, contamination by them should be quite easy.

The data presented here indicate the necessity for the use of every precaution in the collection of gastric samples to avoid contamination. The lowered incidence resulting from improved technique recommends constant reappraisal of methods to further reduce this incidence.

Keltz and associates (9) and Burrows and Barclay (16) have suggested that the occurrence of scotochromogenic organisms is related to previously active tuberculosis or to the chemotherapy thereof. The sporadic and inconstant occurrence of these organisms and the equal incidence in patients and healthy employees argues against any such relationship. It might be suggested that the employees contracted the organism from the patients. This transmission seems unlikely, because, if it occurred, the incidence of true tubercle bacilli in the employees should be much higher since the contact with this organism is greater than with unclassified mycobacteria. Also, *M. tuberculosis* is more resistant to the techniques of processing the specimens.

The finding of one culture of *M. tuberculosis* in a healthy employee was quite disturbing, but this occurrence has been reported (5, 17–19). The low incidence of falsely positive smears in the gastric concentrates of the employees suggests that the diagnostic value of gastric smears should be re-evaluated.

In the Ohio Tuberculosis Hospital, pulmonary disease is occasionally shown to be produced by unclassified mycobacteria, particularly the photochromogens and rarely the scotochromogens. The present data reveal that there is a sporadic inconstant source of unclassified mycobacteria (scotochromogens) which is definitely related to the technique of gastric aspiration but unrelated to the presence or absence of pulmonary disease or antituberculous therapy. These observations suggest that the unclassified mycobacteria are not variants or mutants of *M. tuberculosis*.

Summary

The incidence of unclassified mycobacteria in the gastric contents of healthy employees and patients with pulmonary disease and in the sputum of patients was investigated. The incidence of these organisms in both employees and patients was reduced by refinements in technique of gastric aspiration. The incidence was the same in both groups using the same technique of collection. The data suggest a sporadic inconstant source of unclassified mycobacteria unrelated to tuberculosis or chemotherapy with no evidence to support the theory that these organisms are variants of *M. tuberculosis*.

Sumario

Los Bacilos Acidorresistentes Inclasificados en el Contenido Gástrico del Personal Sano y de los Enfermos de un Hopital de Tuberculosis

Se investigó la incidencia de bacilos ácidorresistentes inclasificados en el contenido gástrico de los empleados sanos y de los enfermos de afecciones pulmonares, y en el esputo de los

últimos. Se rebajó la incidencia de dichos microbios tanto en los empleados como en los enfermos mediante perfeccionamientos introducidos en la técnica de la aspiración gástrica. Usando la misma técnica de colecta, la incidencia de los gérmenes fué idéntica en ambos grupos. Los datos acopiados sugieren la existencia de un foco inconstante esporádico de bacilos inclasificados sin relación con la tuberculosis o la quimioterapia y sin ninguna prueba que apoye la teoría de que dichos microbios son variantes del *M. tuberculosis*.

Resume

Bacilles acido-résistants non classés trouves dans le contenu gastrique de membres du personnel en bonne santé et de malades dans un hopital pour tuberculeux

Les auteurs ont étudié la fréquence de bacilles acido-résistants non classés dans le contenu gastrique d'employés sains, de malades ayant une maladie pulmonaire et dans l'expectoration des malades. La fréquence de ces organismes chez les employés et chez les malades fut diminuée grace à des améliorations apportées dans la technique de l'aspiration du contenu gastrique. La fréquence de ces organismes fut la même chez les deux groupes en utilisant la même technique pour obtenir le contenu gastrique. Les résultats notés font supposer l'existence d'une source inconstante, sporadique de bacilles non classés, n'ayant aucune relation avec la tuberculose ou la chimiothérapie, et n'offrant aucune évidence permettant de soutenir la théorie que ces organismes sont des variétés de *M. tuberculosis*.

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TUBERCULIN SKIN REACTIONS IN MICE HYPERSENSITIZED BY VACCINATION WITH LIVING AVIRULENT TUBERCLE BACILLI[1,2]

ALFRED J. CROWLE[3]

(Received for publication August 7, 1959)

Introduction

Delayed hypersensitivity to tuberculoprotein, or tuberculin allergy, is studied most commonly in guinea pigs, rabbits, and man. Although the importance of mice to tuberculosis research has increased greatly in recent years, the idea that these animals develop little or no tuberculin allergy apparently has prevented their use in connection with this phenomenon. Moreover, this notion has discouraged interpretations of mouse experiment results in the light of possible effects that this kind of allergy might have had upon them. However, this is hardly surprising since until recently data that mice could develop delayed hypersensitivity were equivocal, and after early discouraging reports the problem attracted little attention. Proof gathered in various laboratories during the last five years no longer permits a casual dismissal of the notion that mice do develop tuberculin allergy and that, as in the larger laboratory animals and in man, this allergy may affect the functions of tuberculo-immunity or be instrumental in the pathogenesis of tuberculosis in mice.

Selbie and O'Grady (1) and O'Grady (2) in England, and Gray and his colleagues (3, 4) in Australia, using different techniques and with different strains of tuberculous mice, detected what they interpreted as delayed hypersensitivity to either tuberculin or tubercle bacilli. Experiments recently reported in this country (5–7) support and amplify the findings of these investigators. Mice infected with small quantities of living virulent tubercle bacilli or vaccinated with water-in-oil emulsions of living avirulent bacilli and later challenged intravenously with aqueous suspensions of either type were shown by increases in lung specific gravity to undergo immunologically specific delayed reactions which could not be accounted for on the basis of simultaneously present anaphylactic antibodies (5). Other experiments showed that a delayed type of reaction to tubercle bacilli or their proteins could be detected in any of seven different inbred strains of mice by the techniques of Selbie and O'Grady and of Gray and Jennings, as well as by systemic tests, rectal temperature readings, the lung specific gravity method, and intracutaneous protein injections (6).

The last of these techniques, the tuberculin skin test, as the most widely used method for detecting tuberculin allergy seems to have the greatest influence on opinions with regard to the competence of any animal species to develop the allergy. Thus, consistent failures by investigators to elicit tuberculin skin reactions in mice are the evidence most often quoted to imply that these animals cannot become allergic to tuberculoproteins. However, delayed hypersensitivity to ovalbumin can be induced in these animals (7). It is believed, moreover, that the skin test experiments reported previously (6) and amplified here provide the necessary evidence that mice can develop a classic delayed type of skin reaction to tuberculoprotein.

Materials and Methods

Mice: CF-1white mice of both sexes, purchased for these experiments at six to eight weeks of age from Carworth Farms (New York), were kept in groups of approximately 10, and were maintained on Rockland mouse pellets and water.

Hypersensitization and skin-testing: The mice were hypersensitized with living H37Ra tubercle bacilli grown on slightly modified Kirchner liquid medium; they were challenged with tuberculoprotein prepared from unheated filter-sterilized medium used to grow these bacilli (5). Water-in-oil emulsions of bacilli employed for hypersensitization were made with Arlacel A,[4] *n*-hexa-

[1] From The Colorado Foundation for Research in Tuberculosis, and the Department of Microbiology, University of Colorado School of Medicine, 4200 East Ninth Avenue, Denver 20, Colorado.

[2] Supported by National Science Foundation Grant G-4025, and U.S. Public Health Service Grant E-2689.

[3] James Alexander Miller Fellow of the New York Tuberculosis and Health Association.

[4] Mannide monooleate, a donation of the Atlas Powder Company, Wilmington, Delaware.

decane, and physiologic phosphate buffer at pH 7.4, as described elsewhere (6). Fresh solutions of protein for each series of tests were prepared by dissolving the needed quantity of lyophilized tuberculoprotein in the physiologic sodium phosphate buffer.

Test mice were hypersensitized by two subcutaneous injections of 0.1 ml. water-in-oil emulsion each containing 0.5 mg., wet weight, of H37Ra bacilli. These injections were given a week apart in opposite sides of the groin, and then two weeks were allowed to elapse before tests were begun. Control mice were treated similarly but with water-in-oil emulsion containing no tubercle bacilli.

Skin tests were performed on the shaved flank with 0.01 ml. of 1 per cent tuberculoprotein as detailed in a previous publication (6), but with one exception. In the later experiments (below) mice were anesthetized with ethyl-ether vapor rather than sodium secobarbital[5] before being immobilized with tape on a glass plate for intracutaneous injection. This change in anesthetics lowered the incidence of occasional anesthetic deaths and was presumed to lessen interference by anesthesia with immediate hypersensitivity reactions (8). No differences were noted in delayed hypersensitivity reactions occasioned by this change in technique.

Skin test readings: Delayed skin reactions were read using three measures. The first was millimeters in diameter of visible and/or palpable induration. As mentioned previously (6, 7), no erythema developed in these tests. Reactions equaling or exceeding 3 mm. in diameter were considered positive since rarely did more than one in 10 normal mice skin-tested with tuberculoprotein by this method develop a reaction of this size. The second measure was an estimate of how many times thicker affected skin was than the surrounding normal skin, and the third was a record of whether central necrosis was present. Under the present experimental conditions with the tuberculoprotein system, how meaningful the last measure may be is not yet clear.

Drugs: Two drugs were used for some of the experiments. One was injectable solution of tripelennamine hydrochloride[6] and the other injectable saline suspension of cortisone acetate.[7] Both were diluted appropriately in physiologic salt solution for injection.

Experimental Results

Skin reaction characteristics: It is possible that failure to demonstrate tuberculin allergy in mice, particularly by the skin test, is the result of insufficiently strong hypersensitization techniques. It may be seen in table 1 that mice can be hypersensitized reliably by vaccination with water-in-oil emulsions of living tubercle bacilli. The difference between these test mice and their controls, which received water-in-oil emulsion alone, in skin reactivity measured forty-eight hours after challenge was definite and consistent. The data in table 2, showing results from an average experiment, confirm the consistency of the challenge technique and the regularity of intragroup reactivity; they also illustrate the range and magnitude of reactions which can be expected in this kind of experiment.

Although central necrosis developed on virtually all control animals shown in table 2, it was neither as intense nor as persistent as in test mice; indeed, in some experiments it was infrequent or absent among the former. Apparently, in unsensitized mice, necrosis resulted from injection trauma combined with the moderate toxicity of the particular batch of tuberculoprotein used for experiments described in this paper. This idea has been confirmed by separate similar experiments, using for skin tests another batch of tuberculoprotein prepared similarly but from a different source. This protein injected at 4, 2, or 1 per cent intradermally provoked no necrosis in unsensitized mice and at its greatest concentration rarely was able to induce induration exceeding 1 mm. In hyper-

TABLE 1

Comparison of 48-Hour Skin Test Results Obtained at Different Times from Test and Control Mice in Different Experiments

Experiment	Vaccination Interval*	Mean Induration†		Per Cent Positive‡	
		Test	Control	Test	Control
	weeks				
M 86 H	4	2.6	0.9	60	0
M 93 Hb	4	4.6	1.1	90	10
M 93 Hd	7	3.2	0.3	90	10
M 93 He	4	3.8	1.1	80	10
M 123 H	1	3.6	0.2	100	0

* Time following initial vaccination with living H37Ra tubercle bacilli in water-in-oil emulsion.

† Diameter in millimeters.

‡ Induration diameter ≧ 3 mm.

[5] Manufactured as Seconal by Eli Lilly and Company, Indianapolis, Indiana.

[6] Manufactured as Pyribenzamine by Ciba Pharmaceutical Products, Inc., Summit, New Jersey.

[7] Manufactured as Cortone by Merck Sharp & Dohme, Philadelphia, Pennsylvania.

TABLE 2

RESULTS FROM INDIVIDUAL TUBERCULOPROTEIN SKIN TESTS PERFORMED ON MICE OF A TYPICAL DELAYED HYPERSENSITIVITY EXPERIMENT FOUR WEEKS AFTER THEIR INITIAL VACCINATION

Hypersensitized Mice			Control Mice		
Diameter*	Thickness†	Necrosis	Diameter	Thickness	Necrosis
24-hour readings					
2	2	+	1	tr	+
4	2	+	1	2	+
3	5	+	1	tr	+
3	4	+	1	tr	+
4	4	+	2	tr	+
4	3	+	1	tr	+
2	2	+	3	3	+
2	2	+	2	2	+
6	4	+	2	tr	+
3	4	+	1	tr	+
48-hour readings					
7	5	+	1	2	+
3	3	+	1	2	+
4	4	+	1	tr	+
7	5	+	0	0	−
6	3	+	1	2	+
3	2	+	1	tr	+
5	4	+	1	tr	+
6	4	+	1	tr	+
3	2	+	3	3	+
2	3	+	1	2	+

* Induration in millimeters.

† Times normal skin thickness.

Trace of induration thickness indicated by "tr."

sensitized mice, it regularly caused necrosis at all three concentrations and an average induration of 4 mm. at its two larger concentrations, as contrasted with an average of 0.6 mm. for nonsensitized mice. Neither it nor the more toxic batch of protein induced erythema in either sensitized or unsensitized mice. Photographs of mouse tuberculin skin reactions have been published elsewhere (6).

Induction period: Skin hypersensitivity within groups of vaccinated mice became detectable within a week. In figure 1, this is illustrated by development in these mice of a mean induration diameter of 3.6 mm. forty-eight hours after a tuberculoprotein skin test, compared with an induration of 0.8 mm. in control mice (the latter value, was obtained, for greater significance from the pooled data of several experiments in which control mice were tested identically). For these same sensitized mice, tested one week after vaccination, skin thickness over the test site was approximately twice that in control animals. At two, three, and four weeks after vaccination, as is shown in figure 1, the mean induration for sensitized mice was 5.8, 4.5, and 4.1 mm., respectively. From the second week, attenuation of hypersensitivity seemed to be regular, little remaining beyond the twenty-fourth week when mean induration was only 2.8 mm.

Individual mice in the first experiment were skin-tested only once to eliminate any possible influence that intracutaneous test injections of tuberculoprotein might have on hypersensitivity development. However, tests indicated for the second experiment were done on one group of mice. Since other experiments (unpublished) have shown that the tuberculoprotein skin-testing in this laboratory has little, if any, "boosting" effect on existing delayed hypersensitivity in the mouse or ability to induce this hypersensitivity, the longevity of the hypersensitive state shown in figure 1 does not seem likely to have been a result of these repeated skin tests.

The simultaneous presence of immediate and delayed skin reactivities separable by tempo of appearance after challenge and other characteristics has been observed in mice (7). On the basis of these, the immediate response has been measured as milliliters of diameter of edema developed by the third hour after intracutaneous challenge. This measurement performed periodically on mice of the first experiment in figure 1 showed that, although immediate hypersensitivity to tuberculoprotein developed within one week of vaccination (figure 2), it did not seem to mature as rapidly as delayed hypersensitivity. Reaction intensity to tuberculoprotein in such experiments has never been observed to equal that against egg albumin after similar vaccination (7): petechiae have been absent from the immediate type of reaction to tuberculoprotein. As has been true of the delayed reactions, no observable erythema developed during these immediate hypersensitivity reactions.

Effects of drugs: Cortisone depresses many kinds of inflammatory reaction. Well-known

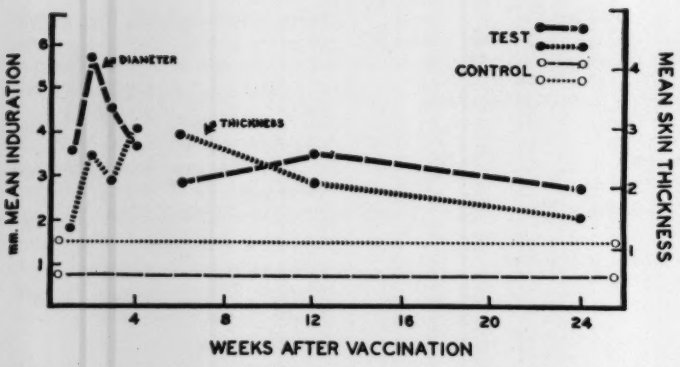

FIG. 1. Mean induration diameter and mean relative thickness of tuberculoprotein skin reactions at forty-eight hours. These tests were performed on mice of two separate but experimentally equivalent experiments, ending for one group four weeks after initial vaccination and beginning for the other six weeks after vaccination. Average reaction levels shown for unsensitized control animals are based on results from a large series of skin tests.

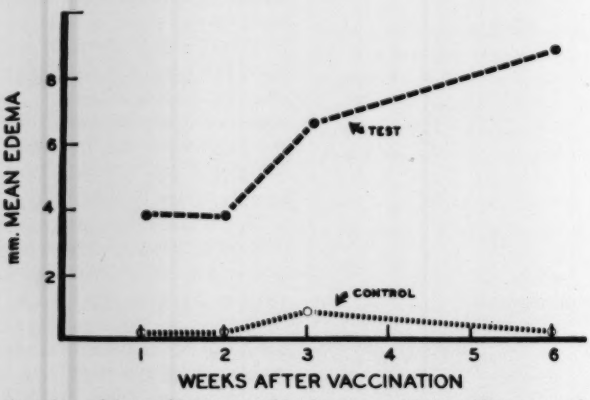

FIG. 2. Growth in mice of immediate-type skin hypersensitivity (diameter of edema read three hours after challenge) to tuberculoprotein with the passage of time after vaccination with living H37Ra bacilli in water-in-oil emulsion.

among these is its effect against delayed hypersensitivity (9, 10). Antihistamine drugs, on the other hand, do not affect the delayed type of reactions (9) but rather inhibit histamine-mediated immediate allergic reactions (11). Therefore, if cortisone could be shown to be effective against the delayed phase of the skin reactions which are being studied in mice and an antihistamine effective against only the immediate (edematous) phase, identification of the two types of hypersensitivity would be more substantially supported. As is shown in figure 3, this is exactly what happened when subjected to study.

In one part of this experiment, a group of hypersensitized mice was divided into two subgroups of 5 each. One group remained untreated with cortisone while, in the other group, each mouse received intraperitoneally 1 mg. of this drug in 0.2 ml. of Tyrode solution thirty minutes before skin testing and again twenty-four hours after challenge. In the second part of this experiment, two other similar groups of hypersensitized mice were used in the same way to study the effects of the antihistamine triplennamine hydrochloride. Treated mice in this part of the experiment received four subcutaneous injections of 0.14 mg. of triplennamine hydrochloride in 0.1 ml. of physiologic phosphate buffer thirty minutes before challenge and sixteen, twenty-four, and forty hours later. For convenience, these treatment

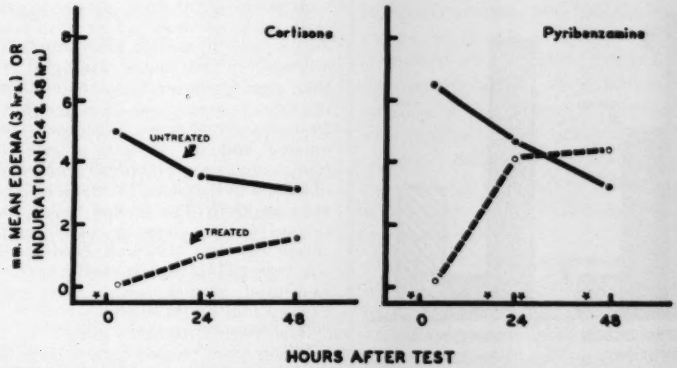

FIG. 3. Effects of cortisone acetate and triplennamine hydrochloride, respectively, on immediate and delayed phases of hypersensitized mouse skin reactions to tuberculoprotein. The immediate phase is represented by three-hour edema measurement, and the delayed by twenty-four- and forty-eight-hour induration measurements. Times of drug treatments are indicated by stars along the abscissas.

times are indicated by stars along the abscissas of figure 3.

The results shown in this figure clearly indicate that cortisone suppressed both immediate (edematous) and delayed (induration) phases of hypersensitive mouse skin reactions to tuberculoprotein, and it is also quite obvious that triplennamine hydrochloride inhibited only the immediate phase. For example, neither cortisone- nor triplennamine hydrochloride-treated hypersensitized mice showed any edema three hours after the skin test, while their untreated controls showed 5.1 and 6.6 mm. of edema, respectively. Untreated mice developed a mean induration of 3.3 mm. forty-eight hours after the skin test while cortisone suppressed this type of reaction to 1.7 mm. Triplennamine hydrochloride had no effect on this delayed reaction: the treated mice developed slightly greater reactions than the untreated mice, mean induration for the two groups being 4.4 and 3.3 mm., respectively.

Specific desensitization: Anaphylactic hypersensitivity can be quelled readily by specific desensitization (12, 13). In the experience of the present writer, a single large dose of tuberculoprotein suffices in mice (5). On the other hand, classic delayed hypersensitivity, whether to tuberculoprotein or to other antigens, is notoriously difficult to suppress by this process (14). This difference between the two types of allergy is useful for differentiating them for, if the immediate type could be abolished while the skin reaction's delayed phase remained unhampered, then the two types of hypersensitivity would be identified by another criterion. It has been shown elsewhere that anaphylactically desensitized mice can react in delayed fashion to intradermally injected tuberculoprotein (6). This observation has been confirmed several times by the present writer. For example, it has been found that hypersensitized mice anaphylactically desensitized with 1 mg. of tuberculoprotein injected intraperitoneally, when challenged twenty-four hours later, showed delayed skin reactivity not essentially different from that in undesensitized controls, even though the mice may have been suffering mild tuberculin shock at the time when they were skin-tested. Although it has also been possible to suppress the delayed type of skin reactivity by desensitization, the following experiment will illustrate how vigorous a contrasting treatment is required for success.

Mice sensitized in the usual manner and proved reactive by tests were injected intraperitoneally every other day with 0.2 ml. of Tyrode solution containing 1 mg. of tuberculoprotein prepared from autoclaved culture medium which had supported growth of virulent human tubercle bacilli (Erdman). Four of these injections were given followed by a fifth of 5 mg. The first three elicited progressively milder systemic tuberculin reactions. The fifth caused a moderate, temporary, and rapidly passing apparently toxic reaction. Four days after the fifth treatment, these test mice and 10 untreated hypersensitive control mice were each skin-tested with tuberculoprotein from the batch routinely used for this purpose, i.e., protein prepared from unheated culture medium which had supported H37Ra growth.

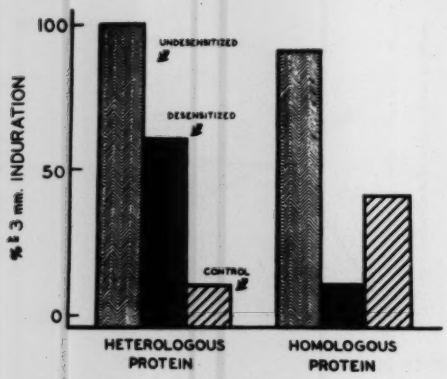

FIG. 4. Per cent of positive skin reactions (3 mm. or more induration diameter forty-eight hours after challenge) in undesensitized mice, desensitized mice, and unsensitized control mice. The same groups of mice tested with heterologous tuberculoprotein were tested three days later with homologous protein, that is, the batch of protein used for desensitization.

Results (figure 4) show that partial desensitization was achieved against what might be judged a rather heterologous antigen. It may also be seen in figure 4 that, when the mice of this experiment were retested with the same kind of protein used for desensitization three days after the first skin test, delayed reactions were more strongly repressed. This second batch of protein apparently was rather toxic since, as may be seen, an unusually large number of unsensitized control mice "reacted," and, as has been noted, 5 mg. injected intraperitoneally at the end of the desensitizing treatment series caused a general toxic reaction. That the desensitized mice were less affected than even unsensitized (essentially normal) control mice suggests that some nonspecific desensitization to the protein's toxicity also was effected.

Passive transfer: The best criterion presently available for differentiating delayed hypersensitivity reactions from immediate reactions is to demonstrate that the delayed reactivity can be transferred passively from vaccinated to normal animals with lymphoid cells but not with serum, and that the immediate reactivity is transferred with serum but not with cells. Since this procedure has been applied successfully to distinguishing these types of skin hypersensitivity to ovalbumin in mice (7), it now was attempted with the tuberculoprotein system.

A group of 10 mice shown hypersensitive by tests made six days before were used as immune donors, and 10 normal mice supplied control cells and serum. Each mouse was lightly etherized and then exsanguinated from severed armpit vessels, the blood from mice of a group being pooled. Immediately after each was bled, its rib cage was opened and its thymus glands and attached lymph nodes were removed and pooled with those of others in its group in 10 ml. of Tyrode solution kept at 37°C. The pooled blood was allowed to retract for one hour at body temperature; the serum was then drawn off. Each of 10 normal mice was injected intraperitoneally with 0.4 ml. of this undiluted pooled serum and was skin-tested twenty-four hours later.

The pooled thymus glands in their Tyrode solution were poured into a large medium-grind Ten Broeck tissue grinder and homogenized by two slow twisting piston excursions. The homogenate, poured from the grinder into a centrifuge tube, was centrifuged at 500 g. for fifteen minutes, and most of the resulting supernatant fluid was drawn off and discarded, leaving 4.5 ml. of the original volume, in which the sediment then was resuspended gently. The suspension was injected in 0.4 ml. aliquots intraperitoneally into 10 normal recipient mice which were skin-tested forty-eight hours later. Approximately two hours elapsed from the time the first donor was killed and the last recipient was injected.

The first experiment (figure 5) suggests that mice which received either serum or cells from hypersensitive donors may have had slightly more delayed skin reactivity to tuberculoprotein

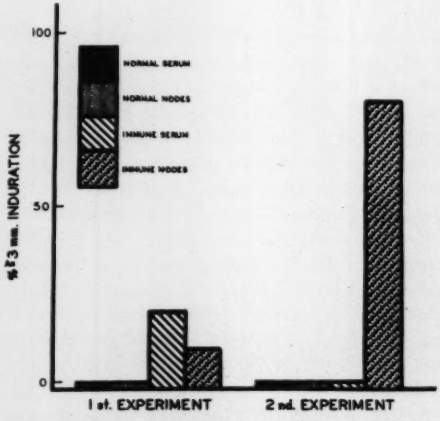

FIG. 5. Per cent of positive skin reactions (3 mm. or more induration forty-eight hours after challenge) in two passive transfer experiments in mice receiving serum or lymphatic tissue ("nodes") from normal or immunized mice.

than those receiving normal cells or serum. However, since, in any set of normal animals being skin-tested, the technique used often causes one mouse in ten to "react" (3 mm. skin induration), the differences between mouse groups in this passive transfer experiment hardly could be meaningful.

Since this experiment had failed, some modifications in technique were made for another attempt to passively transfer tuberculin allergy in mice.

A group of 10 mice from the same initial series of animals was used as donors. They were skin-tested one week before being used to confirm their hypersensitivity. The quantity of transferred tissue was approximately doubled by using only 5 recipients, although the fluid volume injected was the same. Tissue homogenate was prepared in the same way. Serum from immunized and nonimmunized mice was obtained as in the previous experiment except that the clot was allowed to retract overnight in the refrigerator; each recipient was injected with 0.6 ml. of undiluted serum instead of the 0.4 ml. used before. Again, cell recipients were skin-tested forty-eight hours—and serum recipients twenty-four hours—after passive transfer. However, special precautions were taken to obtain maximal significance from the skin tests. All recipients were tested at the same time with the same protein solution and the intracutaneous injections were performed with utmost care to minimize nonspecific reactions. Instead of the 0.01 ml. usually injected, 0.015 ml. was employed.

As the second experiment (figure 5) clearly shows, mouse recipients of immune thymus cells reacted specifically to tuberculoprotein, while mice receiving normal cells or serum from either normal or hypersensitive mice uniformly were unreactive. This finding that mice can be made passively hypersensitive to tuberculoprotein by transfer of thymus cells, but not serum, has been repeated in separate experiments, but only when the donor-recipient ratio is at least as great as was used in the first experiment reported here. Such results, of which figure 5 is a particular illustration, supply further evidence that mice develop classic tuberculin allergy detectable by skin tests.

Discussion

The observations presented confirm the belief that appropriate skin tests can detect tuberculin allergy in properly hypersensitized mice. Skin reactions developed with the same tempo as in the larger animals and had the same appearance except for their lack of erythema. They could be prevented with cortisone but not with triplennamine hydrochloride. Hypersensitivity responsible for these reactions could be transferred passively with lymphoid cells but not with serum, and it could be diminished by specific desensitization, but only by treatments considerably exceeding in intensity those needed to abolish immediate hypersensitivity.

As in previous experiments utilizing ovalbumin antigen (7), tuberculoprotein injected intradermally into hypersensitized mice provoked both immediate and delayed phases of reactions due, respectively, to anaphylactic and delayed hypersensitivities. These were distinguishable not only by their times of development but also by all the criteria just mentioned. Arthus reactions have not been observed in the present experiments with tuberculoprotein, although precipitating antibodies to this antigen have been detected in some of the mice. In contrast, the experience (unpublished) of the present investigator with the apparently much stronger antigen ovalbumin shows that these animals develop strong precipitating antibodies to it which apparently cause Arthus skin reactivity. In prolonged experiments, the Arthus reactions developed by these mice physically obscure true delayed hypersensitivity reactions.

Three subjects concerned with the present experiments deserve special discussion. The first has to do with the suppressive effects of drugs on immediate and delayed skin reactions. The second pertains to specific desensitizing characteristics of delayed hypersensitivities and introduces a supplementary discussion and comparison of classic tuberculin allergy and the type of delayed hypersensitivity recently investigated in some detail by Uhr and associates (15). The third subject is the probable importance of delayed hypersensitivity in tuberculous pathogenesis in mice and how it may affect tuberculo-immunity observed in these animals.

Utilizing the effects of various drugs to differentiate immediate from delayed hypersensitivity is at best risky, since some drugs can suppress both types of reaction while others specific for one kind may act only in selected animal species or may affect one type of immediate hypersensitivity but not another. Moreover, the results can be confused by superposition of several types of hypersensitivity upon

each other. Nevertheless, under fortunate conditions, such as obtained in the present experiments, this method of differentiation can be useful. Also, its use also yields information helpful to understanding hypersensitivity mechanisms.

That cortisone inhibited both immediate and delayed phases of the tuberculoprotein skin reaction in mice agrees with what is known about its effects against inflammation. It has a well-documented suppressive action on the tuberculin skin reaction in the larger animals and in man (9, 10). Although cortisone often is thought to be unable to ameliorate immediate hypersensitivity reactions, is said to be ineffective against anaphylaxis in guinea pigs (16, but *see* 17), and cannot prevent passive cutaneous anaphylactic reactions in these animals (11, 18), it does suppress mouse anaphylaxis (19). It has been reported to counteract edema in the Arthus reaction (20). By precedent, then, cortisone might be expected to inhibit both phases of the skin reaction. It may have acted as a general anti-inflammatory agent (9, 10) by its slight antihistaminic activity (20) or by inhibiting some event in a chain of events leading indirectly to later injurious tissue changes and originally provoked by antigen-antibody combination (16).

Currently, histamine is not thought to be primarily responsible for the immediate type of reaction in mice because these animals natively are so resistant to it. This point is set forth well by Malkiel and co-workers (21, 22) who also propose, however, that antigen-antibody reactions may liberate small amounts of histamine which could supplement or trigger other anaphylactic mechanisms in mice (21). Hence, this liberation, which might occur intracellularly and thus be easily overlooked (11, Benacerraf in 23), could initiate or take vital part in a series of reactions culminating in anaphylactic shock (24). Cameron's recent work (24), showing that bovine gamma globulin-induced mouse anaphylaxis can be almost totally prevented with the antihistaminic mepyramine maleate (Anthisan®) demonstrates that antihistaminics and perhaps histamine may have unexpectedly important roles in mouse anaphylactic hypersensitivity reactions. In other animals, anaphylaxis and passive cutaneous anaphylaxis can be suppressed with antihistaminics (11), but the Arthus reaction may not be (11, 23).

Evidence from other laboratories does not necessarily conflict, then, with the present findings that the antihistaminic triplennamine hydrochloride inhibitis what appears to be a local anaphylactic skin reaction in mice. However, Malkiel and Hargis (22) have reported that, although triplennamine hydrochloride inhibited histamine shock in their mice, it did not inhibit anaphylactic shock. They used as much as four times the drug quantity used in the present study and an entirely different antigen-antibody system. Perhaps the systemic anaphylactic reaction involving either more tissue or tissue with a different kind of response to antigen-antibody reactions may be suppressed by entirely different doses or types of drugs than are necessary for local reactions. What antigen-antibody system is used also may have considerable importance.

As in other animals, anaphylactic hypersensitivity in mice can be suppressed quite easily by specific desensitization (5, 12, 13); recovery from anaphylactic shock makes the mice refractory to subsequent antigen injections. Results reported here suggest that mouse tuberculin allergy yields to specific desensitization but only upon the same persistent treatment required in the larger animals (14). Thus, this is dissimilar to either immediate hypersensitivity or the type of delayed hypersensitivity described by Uhr and associates (15) which apparently can be abolished by specific desensitization as easily as anaphylactic hypersensitivity. With respect to the latter, the significance of the finding previously reported (6) and confirmed in present experiments should be emphasized i.e., that mice anaphylactically desensitized retain the delayed type of skin reactivity. This reactivity's development does not seem to be a phase in the growth of immediate hypersensitivity, as suggested by Pappenheimer and Freund (15). For, although in the present experiments the delayed hypersensitivity apparently "matured" more rapidly than the immediate, it remained essentially undiminished while immediate hypersensitivity continued development, apparently independently. Hence, the present writers would agree with the opinion (25) that perhaps more than one basic type of delayed hypersensitivity exists.

The pathogenesis of tuberculosis in man and in most animals is described with frequent references to delayed hypersensitivity, particularly

for the role this allergy has in provoking tissue destruction characteristic of it (14). However, only recently (3, 4) has it been possible to implicate this allergy in the development of mouse tuberculosis because these animals generally have been thought not to develop it. Revising present views on this subject, in the light of knowledge that they do, may permit clarification of some yet inadequately explained observations made on tuberculosis and similar diseases in these animals.

For example, Sever and Youmans (26) made the intriguing observation that the tubercle bacillary population in the lungs of mice with chronic tuberculosis attains a certain level and then becomes constant, neither increasing nor decreasing significantly. Yet, these mice eventually die of tuberculosis from "respiratory insufficiency," in which their lungs have become congested and edematous and "show massive infiltration by mononuclear cells" (26, 27). In some of the unpublished experiments from this laboratory, it has been found that mice that died after more than a year's infection with tubercle bacilli showed enlarged, congested, and hemorrhagic lungs without grossly visible tubercles in which only moderate numbers of poorly staining granular tubercle bacilli could be found. The lungs of mice dying from chronic tuberculosis bear a striking resemblance to those of mice killed by, or during, tuberculin shock (5). Perhaps, then, death of the chronically tuberculous mouse is precipitated by delayed hypersensitivity reactions between its lungs and persisting tubercle bacilli. If this proves true, then one aspect of tubercle bacillary virulence for mice would rest in bacillary capability to remain in the mouse lung long enough and in sufficient numbers to elicit fatal delayed hypersensitivity incapacitation of the lungs. This aspect might also be of some importance in mouse death from acute tuberculosis since the writer has shown that tuberculin allergy can develop within as little as a week after vaccination. The effectiveness of acquired tuberculo-immunity, then, might very well depend in mice upon how greatly growth of the bacillary population can be curtailed, since the greater it is the more vigorous might be the victim-parasite allergic reaction and consequent lung destruction.

Summary

Mice vaccinated subcutaneously with water-in-oil emulsions of living, avirulent tubercle bacilli (H37Ra) developed immediate and delayed hypersensitivities to tuberculoprotein detectable and distinguishable by skin tests performed with this antigen. The former, an anaphylactic type of hypersensitivity, could be suppressed readily by specific desensitization or by treating the animal with tripelennamine hydrochloride (Pyribenzamine®) or cortisone. The immediate reaction was a rapidly developing edematous skin swelling which subsided before the reaction due to delayed hypersensitivity had developed significantly. The latter—classic tuberculin allergy—could be specifically suppressed only by a series of desensitizing injections. It was not affected by tripelennamine hydrochloride but was counteracted by cortisone. The delayed reaction in the skin developed several hours after challenge and was characterized principally by induration and necrosis. The delayed hypersensitivity was transferred passively from hypersensitive to normal mice with thymus-gland and lymph-node homogenates but not with serum.

Sumario

Cutirreacciones a la Tuberculina en Ratones Hipersensibilizados por la Vacunación con Bacilos Tuberculosos Avirulentos Vivos

Los ratones vacunados subcutáneamente con emulsiones de bacilos tuberculosos avirulentos vivos (H37Ra) en agua-en-aceite manifestaron hipersensibilidades inmediata y tardía a la tuberculoproteína, descubribles y distinguibles con cutirreacciones ejecutadas con dicho antígeno. La primera, forma anafiláctica de hipersensibilidad, puede suprimirse fácilmente por la desensibilización específica o tratando el animal con enamina-triple (Piribenzamina®) o cortisona. Prodúcese así una hinchazón cutánea edematosa de rápido desarrollo que cede antes de que tome mayor auge la reacción debida a la hipersensibilidad retardada. La última—clásica alergia tuberculínica—puede suprimirse únicamente con una serie de inyecciones desensibilizantes. No la afecta la enamina-triple, pero la contrarresta la cortisona. Ocasiona la misma una cutirreacción que aparece varias horas después de la provocación y se caracteriza principalmente por induración y esfacelo. La hipersensibilidad retardada

fué transmitida pasivamente de los ratones hipersensibles a los normales con homogeneatos del timo y de los ganglios linfáticos, pero no con el suero.

Resume

Cutiréactions à la tuberculine chez des souris sensibilisées par vaccination avec des bacilles tuberculeux vivants, non virulents

Des souris vaccinées par voie sous-cutanée avec des émulsions d'eau et d'huile de bacilles tuberculeux vivants, non virulents (H37Ra) développèrent une hypersensibilité immédiate et retardée vis à vis des protéines tuberculiniques, ce qui fut détecté et étudié par des réactions cutanées produites par cet antigène. La première réaction du type anaphylactique d'hypersensibilité peut être supprimée surement par désensibilisation spécifique, ou en traitant l'animal par la triple-ennamine (Pyribenzamine®) ou par la cortisone. Cela cause un édème cutané qui se développe rapidement et persiste avant que la réaction due à l'hypersensibilité retardée ne s'installe nettement. La dernière réaction—classique allergie à la tuberculine—ne peut être supprimée d'une façon spécifique par une série d'injections désensibilisantes. Elle n'est pas affectée par la triple-ennamine, mais est enravée par la cortisone. Elle cause une réaction cutanée qui se développe plusieurs heures après et est caractérisée principalement par une induration et de la nécrose. L'hypersensibilité retardée fut transferrée passivement de souris hypersensibilisées à souris normales au moyen de préparation homogène de thymus et de ganglions lymphatiques, mais pas avec du sérum.

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SUCCESS OR FAILURE IN TUBERCULOSIS TREATMENT AND CONTROL[1, 2]

Some Psychologic Aspects

GEORGE M. ECKLEY AND HARRY R. DRAPER

(Received for publication November 13, 1959)

INTRODUCTION

The medical and surgical treatment of tuberculosis has improved considerably in the past ten to fifteen years; so much so, in fact, that the average case of active disease, diagnosed promptly, in a patient who accepts hospital care and follows the recommendations of his physician stands a very good chance of becoming inactive in a relatively short period of time and remaining that way indefinitely.

Yet, in this institution, a state-supported tuberculosis hospital located in a large industrial city, a fair number of patients today are becoming chronic, infectious, cavitary cases because of their indifferent attitude, which manifests itself by irregular treatment, refusal of treatment, and premature discharge. This seems to be a crucial factor in tuberculosis control, especially in the large industrial urban community.

Since this hospital admits transfers from other city hospitals, i.e., patients too ill to travel to tuberculosis hospitals in rural areas, treatment failures, and, in general, patients from the lower socio-economic levels, at first it was assumed that the problem was merely a matter of education, and an educational course in the disease was set up for the patients. This course was given in sessions of one hour a week. Every attempt was made to make it as interesting as possible by setting up microscopic slides of sputum positive for tubercle bacilli, showing sputum cultures and susceptibility studies, exhibiting regular and body section roentgenograms, as well as by demonstrating pathologic sections of cavities which had been obtained by surgical resection. The attendance at the first session was well over 90 per cent of the patients in the hospital. This dwindled within a few weeks to perhaps a dozen patients who lasted through most of the course; but a few weeks before the end of the course it was abandoned because all patients stopped attending.

Finally, the conclusion was reached that the problem was not the medical or surgical treatment of tuberculosis or the intellectual capacity or store of knowledge regarding the disease in the patient's mind, but rather the particular personality of the patients involved. Accordingly, group psychotherapy was started at first with the patients; later this was extended to include meetings at regular intervals with the staff. While this undoubtedly improved the general situation and helped the patients who attended, the problem that evolved was that only a few patients of the total population would accept this form of treatment. The group approach seemed to be somewhat better with presurgical patients than with the patients receiving medical treatment.

However, since the vast majority of patients refused group psychotherapy, it was thought that more should be learned about the personality of the total patient population as a group. At first an attempt was made to use the Madison Sentence Completion Form. Again resistance was encountered, and most of the patients refused to complete the form. At this point the individual approach to each patient was decided on.

METHOD OF INVESTIGATION

Accordingly, to gain some insight into this problem, one of the writers, a psychiatrist (H. R. D.), interviewed 100 patients consecutively admitted to the hospital between November 16, 1956, and November 18, 1957, to try to obtain a cross-section profile of the personalities in the hospital. This interview was arranged as soon after admission as possible. It was also possible for the psychiatrist to see 57 of these patients on one or more additional occasions. These patients were all males, as this hospital does not admit female patients.

[1] From the Henry R. Landis State Hospital, Department of Health, Commonwealth of Pennsylvania, Philadelphia, Pennsylvania.

[2] Presented, in part, before the Joint Meeting of the Pennsylvania Trudeau Society and the Pennsylvania Chapter, American College of Chest Physicians, Scranton, Pennsylvania, April 16, 1959.

Characteristics of the Various Groups

Since the primary interest of the writers was to see how the patients reacted to active tuberculosis, they broke them down into various groups. These included the so-called "normal" group of 24, a personality-disorder group of 26 (these consisting of all personality disorders with the exception of the sociopathic personalities), an alcoholic group, and a final group of 15 sociopathic personalities. The sociopathic patients were split off from the remainder of the personality-disorder group, as their behavior seemed basically different from that of the rest of the group.

In an attempt to simplify the problem, only three groups will be discussed and compared: the so-called normal, the personality-disorder group (exclusive of the sociopathic group), and the sociopathic group. The alcoholic group will not be discussed individually, as the behavior of these patients falls between that of the personality-disorder group and the sociopathic group. This can be best seen in figures 2 and 3.

In an attempt to verify the findings, as many as possible of these patients were interviewed one or more times. This was possible with 57 of the patients. The psychiatrist made no change in his original impression of 47 of the patients. Two other patients, who had had no significant problems on admission, later developed psychoneurotic disease (conversion reaction and depressive reaction), both apparently on the basis of additional stressful situations which appeared later in the course of their illness. Three had their diagnoses slightly changed or refined, such as a change from the alcoholic to the sociopathic group. Three were changed from the so-called normal group to the passive dependent-personality group.

The only large error occurred in patients who were originally called "no significant psychiatric problem," but who during their stay in this hospital evidenced repeated episodes of alcoholic abuse, ending in conflict with other patients, with the hospital authorities, or with the police. These were reclassified into the alcoholic group. The status of the patients was evaluated approximately one year after the completion of the interviewing, and the results of treatment were assessed.

Results of Interviews

The final breakdown of these 100 patients from a diagnostic standpoint is given in table 1. The miscellaneous group in table 1 was an assorted group made up of small numbers of various diagnostic classifications, and these are given in table 2. The impression of no significant psychiatric problem which may be referred to as a so-called normal group implies that, while the patient may have had symptoms produced by emotional conflict at the time of the interview, they were appropriate to the situation in which the patient found himself. The group called passive-dependent personalities were, diagnostically speaking, passive-aggressive personalities of the passive-dependent type.

TABLE 1

Primary Psychiatric Diagnosis of 100 Consecutive Admissions

Diagnosis	Number of Patients
No significant psychiatric problem	24
Chronic alcoholism	18
Sociopathic personality	15
Passive dependent personality	13
Inadequate personality	8
Miscellaneous	22
Total	100

TABLE 2

Primary Psychiatric Diagnosis of Miscellaneous Group

Diagnosis	Number of Patients
Organic brain syndrome*	8
Depressive reaction	3
Mental deficiency	2
Schizoid personality	2
Situational anxiety	2
Immature personality	1
Paranoid personality	1
Conversion reaction	1
Chronic anxiety reaction	1
Emotionally unstable personality	1
Total	22

* Various etiologies, i.e., syphilis, alcoholism, and arteriosclerosis

Particularly noticeable was the high proportion of abnormal subjects, especially in the total of personality disorders (constituting 59 per cent of the entire group), and the low proportion of those given a clean bill of health (only 24 per cent) from the emotional point of view. This is best seen in figure 1. Another observation in the miscellaneous group was the small proportion of psychoneuroses, representing only about 7 per cent of the group.

There were some in the group who manifested their personality disorders by drinking. This group included all of the alcoholics plus many others with basic diagnoses of various personality disorders plus a few of the organic brain-

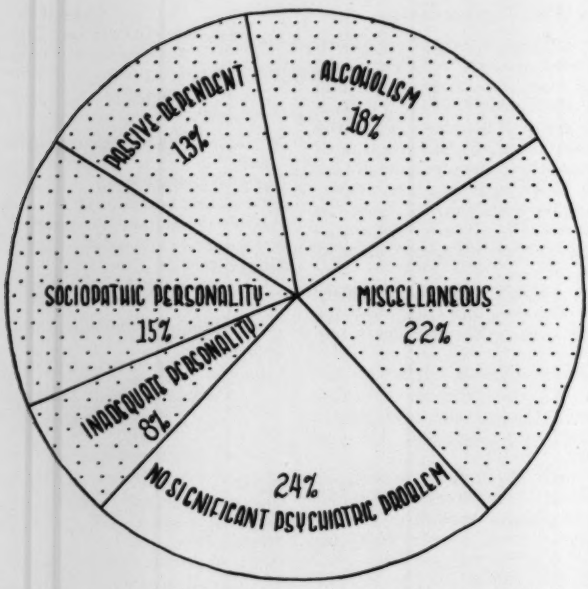

FIG. 1. Diagram showing the relative proportions of the major personality groupings.

syndrome group, the result of alcoholism. Thirty-five per cent of the patients exhibited severe manifestations of repeated alcoholic abuse.

Normal group: The normal group was comparable with the general hospital population on the basis of age, sex, and color. The number of far advanced and moderately advanced cases were equal, which means that the disease in this group was less far advanced on admission than that of the general hospital population.

The main thing observed about this group was that at the time of follow-up study, 58 per cent had been discharged with medical consent; only 17 per cent were still in the hospital. The other observation was that in this group 71 per cent (or 2 of 3 patients) in whom pulmonary surgery was recommended accepted it. In other words, the majority of so-called normal patients accepted treatment, got well, and went home within a reasonable length of time.

Personality-disorder group: The personality-disorder group was primarily different in character from the normal group in that the ratio of white to nonwhite was approximately 3:2 (in the general hospital population it was 1:1), and in the high percentage of single persons, 38.5 per cent versus 25 per cent in the normal group, with only 4 per cent of this group married as opposed to 46 per cent in the normal group. Interestingly, this group also reacted differently to tuberculosis. Whereas the patients in the normal group tended to get well and go home, the personality-disorder group showed 54 per cent still in the hospital at the time of the follow-up study, only 19 per cent having been discharged with medical consent. Another point of interest is that the premature discharge rate in this group is not appreciably greater than in the normal group, i.e., 17 per cent versus 19 per cent.

Another difference in the personality-disorder group was their reaction to surgery, as only 18 per cent (or less than one in 5) accepted pulmonary surgery when it was recommended. In other words, the characteristic of this group as a whole was that it tended to be composed of lone individuals who were more likely to be white than nonwhite. They did not leave against medical advice, they accepted treatment indifferently, and tended not to get well, but to stay on for a prolonged hospital stay.

Sociopathic group: The sociopathic group was characterized by a younger group (on the average, ten years younger than the general hospital population) and in the extent of their disease

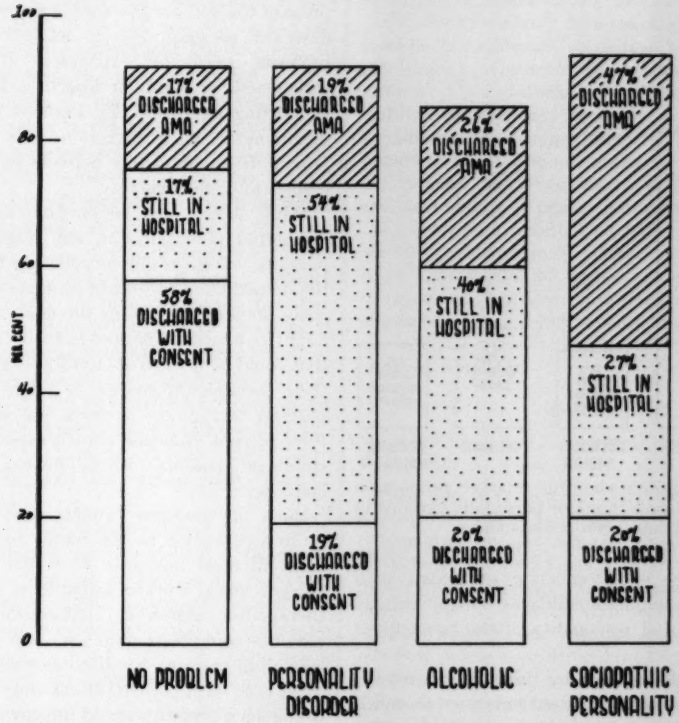

FIG. 2. Diagram showing the relative proportion of results of treatment of major personality groups at the time of the follow-up study. Percentages not shown on the graph represent deaths and transfers which were not great enough to be significant.

which was much less on admission (only 27 per cent of this group had far advanced disease) than that of the general hospital population (in which 67 per cent had far advanced disease). Their characteristics in regard to their disease were their high rate of premature discharges (47 per cent) and their high refusal rate for surgery (only 20 per cent or one in 5 accepted). In other words, while they were a younger group with less extensive disease and a good prognosis, their impulsiveness and refusal to follow the advice of authority probably precluded a good final result. The discrepancy of adequate treatment results between the groups can best be seen in figures 2 and 3. It can also be noted in these figures that the alcoholic group refused surgery to approximately the same degree as the personality-disorder group and the sociopathic group, while their premature discharge rate and their failure to improve fell between the other two groups.

One of the results of the study, which does not readily lend itself to measurement, was the feeling by most of the hospital personnel that the interviewing of each new patient by the psychiatrist seemed to have a beneficial and tranquilizing effect on the hospital population as a whole. Optimal acceptance of the psychiatrist by the patient is contingent upon making his interviews a routine part of the over-all admission study of the patient.

DISCUSSION

There is no doubt that since 500 B.C. there have been repeated suggestions that emotional stresses play a part in the development and course of tuberculosis (1–5). There has been a wealth of material describing the tuberculous personality, and this frequently describes a

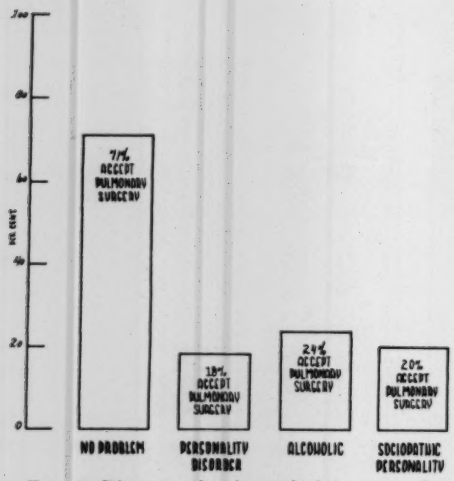

FIG. 3. Diagram showing relative proportion of surgical refusals in major personality groups in which surgery had been recommended.

"high" degree of dependency associated with difficulty in handling hostility as central features in the premorbid personality of the tuberculous patient (6–9).

The breakdown made by this hospital, revealing that 21 per cent of the patients were classified primarily in the passive-dependent or inadequate-personality group, would seem to confirm the previously noted dependent nature of many tuberculous patients. In terms of this study, excessive dependency and passivity seemed to be an ill omen in the prognosis of the disease. The sociopathic group, constituting 15 per cent, revealed an ineffectiveness and awkwardness in handling hostile impulses which, resulting in high self-discharge and surgical refusal rates, militated against a successful result from treatment.

The high percentage of personality disorders (59 per cent) was a very discouraging finding, for patients with personality disorders have relatively poor prognoses from the psychotherapeutic standpoint. However, there is a moderate amount of material suggesting that, more often than can be accounted for by chance, there is some relatively severe emotional upheaval preceding the onset of the tuberculosis and perhaps "triggering off" or precipitating this disease as well as other illnesses (6, 10, 11). This upheaval, which precedes the disease and seems to prepare the soil for the seed, has been variously described as grief, loss of self-esteem, anxiety, repressed needs for expression, and the real, threatened, or symbolic loss of a loved one or supporting figure (6, 12). Perhaps the common denominator is a situation with which the patient feels completely helpless to cope in any realistically effectual way.

While it may not be possible to change the premorbid personality in any large number of patients, efforts could conceivably be limited to the immediately preceding conflicts which apparently prepare the soil for the acute disease. This might be a vulnerable spot in their "iron curtain" that could be worked on, letting them "ventilate" their feelings. Moreover, it might be possible perhaps to indicate to them the way in which their present situation reverberates with similar childhood trauma, when, indeed, they were helpless.

Such a program would probably require quite an addition to the Social Service Department in most hospitals. It would require psychiatric social workers capable of working in a therapeutic case-work relationship to these patients and their families, and also require enough of them to spend at least several hours a week with each patient. It can only be suggested that such a program would improve the patient's ability to accept treatment and get well. The high cost of treatment failures in our society suggests that the program might, in the long run, prove economically sound and represent a real saving to society (13).

It might also be possible, with some suggestions from our psychiatric colleagues, to change the milieu or climate of the hospital so that the patient with a character or personality disorder (especially the sociopathic personality) might feel more comfortable and at home in the hospital, thereby decreasing the present high premature discharge rate.

SUMMARY

Even though adequate medical and surgical care is available for pulmonary tuberculosis, a fairly high percentage of patients do not achieve satisfactory arrest of their disease. While adequate knowledge of the disease by the patient is essential, it does not seem to be the factor involved in the failure to get good results.

The writers believe that the foregoing findings

suggest rather strongly that the patient's personality has much to do with the way he handles his disease, and that treatment failure seems to be associated with the high proportion of personality disorders (59 per cent or approximately 3 of every 5) seen in the present hospital population. To be realistic, the fact must be accepted that personality disorders, by definition, are not likely to show much improvement with anything less than intensive, prolonged, and individual psychotherapy. This does not mean that group psychotherapy is useless, for it has a definite place and should be used whenever possible. However, it is doubted that any exceptional results will be obtained by any measure less than intensive individual therapy. Additional supplementary help might be obtained by controlling the emotional climate of the hospital so that the patient with a character disorder feels more comfortable in, and less antagonistic toward, the institution.

The routine interviewing of each new patient by the psychiatrist seemed to have a beneficial effect on the hospital population as a whole, but considerably more personnel trained for individual counseling will probably be necessary if the remaining "hard core" of tuberculosis is to be stamped out in this country.

Sumario

El Exito o el Fracaso en el Tratamiento y Dominio de La Tuberculosis (Algunos Aspectos Psicológicos)

Aunque hay a mano adecuada asistencia médica y quirúrgica para la tuberculosis pulmonar, un porcentaje bastante elevado de los enfermos no obtiene estacionamiento satisfactorio de su dolencia. Si bien es indispensable un conocimiento adecuado de la enfermedad de parte del paciente, no parece que éste sea el factor que interviene en la falta de buenos resultados.

Opinan los AA. que los hallazgos precitados sugieren bastante elocuentemente que la personalidad del enfermo tiene mucho que ver con la forma en que atiende a su afección y que el fracaso terapéutico parece asociarse con la alta proporción de trastornos de la personalidad (59 por ciento o aproximadamente 3 de cada 5) observada en la actual población del hospital. Para ser realistas, hay que aceptar el hecho de que los trastornos de la personalidad, por su misma definición, no son susceptibles de mostrar mayor mejoría con nada que no sea psicoterapia intensa, prolongada e individual. Esto no significa que la psicoterapia colectiva sea inútil, pues tiene su puesto bien definido y debe usarse siempre que sea posible. Sin embargo, es dudosa la obtención de resultados excepcionales con ninguna providencia inferior a la intensa terapéutica individual. Cabría obtener mayor ayuda complementaria acondicionando el clima afectivo del hospital de modo que el enfermo que padezca de un trastorno del carácter se sienta más a sus anchas en el establecimiento y menos antagónico al mismo.

La entrevista habitual de cada enfermo nuevo con el psiquíatra pareció ejercer efecto beneficioso sobre la población del hospital en conjunto, pero se necesitará probablemente mucho más personal adiestrado en el asesoramiento individual si va a erradicarse en este país el restante "duro núcleo" de la tuberculosis.

Resume

Succès ou échec dans le traitement de la tuberculose. Son controle. Quelques aspects psychologiques

Bien que le traitement chirurgical et médical convenables soient possibles pour la tuberculose pulmonaire, un pourcentage passablement élévé de malades n'arrivent pas à un arrêt satisfaisant de leur maladie. Bien qu'une connaissance convenable de la maladie soit essentielle pour le malade, il ne semble pas que ce soit le facteur que l'on puisse considérer dans l'échec à obtenir de bons résultats.

Les auteurs croient que les données ci-dessous font fortement penser que la personnalité du malade a beaucoup à faire dans la façon dont il dirige sa maladie et que l'échec du traitement semble assoçié à une forte proportion de troubles de la personnalité (59% ou à peu près 3 sur 5 malades) rencontrés dans la population présente de l'hôpital. Pour être dans le vrai, on doit accepter le fait que les troubles de la personnalité, par définition, ne montrent pas d'amélioration avec aucun traitement à part une psychothérapie individuelle, intensive et prolongée. Ceci ne veut pas dire que la psychothérapie de groupe est inutile, car elle a sa place définie et devrait être utilisée autant que possible. Cependant il est douteux que des résultats exceptionnels soient obtenus par un traitement autre qu'une psychothérapie individuelle intensive. Une aide supplémentaire peut être ajoutée en contrôlant l'ambiance émotionnelle de l'hôpital en sorte que le malade ayant des troubles de la personnalité s'y sente plus à l'aise et soit moins opposant vis à vis de l'institution.

L'entretien systématique de chaque nouveau malade avec le psychiatre semble avoir un bon effet sur toute la population hospitalière, mais beaucoup plus de personnel habitué à guider

individuellement le malade sera probablement nécessaire si "le noyau dur" de tuberculeux restant dans ce pays doit en être banni.

Acknowledgment

The figures for this article were kindly produced by Miss Dorothy Baird, O.T.R.

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WARD SOCIALIZATION AND ADJUSTMENT TO THE TUBERCULOSIS HOSPITAL[1, 2]

RUSSELL P. NORMAN[3]

(Received for publication September 14, 1959)

Introduction

Until recently, most studies on the response to hospitalization for tuberculosis could be classified into two groups: those which have emphasized personality variables (1–3) and those which have utilized personal data or demographic variables (3–5). A smaller number of studies have concentrated on other factors such as intelligence (6–7) and vocational preference (8). However, in nearly every instance, none of these approaches has contributed significant findings. The behavioral measure of response to hospitalization is usually the type of discharge a patient receives. However, in comparing groups of patients receiving regular and premature discharges, few factors have been found which distinguish the two, and the meager findings that have turned up have seldom been replicated.

Although most workers recognize that premature discharge is much too complex a problem to be pinned down to one factor, the present writer believes that some of the most important variables have been almost completely neglected. To quote from Barker and associates (9):

> The practice of dealing with patients in face to face groups within the sanatorium provides a social situation with great potentialities for both favorable and unfavorable adjustment of patients. It is apparent that sanatoriums vary greatly in the way they handle this situation. (page 174)

Thus, the patient who resorts to premature discharge may be one who is unable to form adequate social relationships while in the hospital. His inability to interact with others and their rejection of him combine to accentuate the stressful nature of the situation. It may be presumed that the well adjusted patient is one whose interpersonal relationships are characterized by adequacy and stability both inside and outside the hospital.

The method chosen here to evaluate interpersonal behavior within the hospital is sociometry, a social psychologic investigating technique developed by Moreno (10). The particular application of this method will be explained later but, briefly, it has as its aim the discovery of the feelings of group members toward each other by asking each to choose those with whom he would most and/or least like to engage in some activity. This activity is referred to as the "sociometric criterion" and can be anything associated in some way with social behavior and companionship, such as playing a game, working at a particular task, or sharing a dormitory. The term "sociometric status" then refers to the response of the group to one particular member. If he is chosen often and is seldom or never rejected, he is said to have a high sociometric status. Schauer (11) has outlined the advantages of using sociometric techniques in a hospital setting.

In most cases the patient, barring activity class restrictions, is allowed to associate with whom he pleases, and those most readily available for this type of interaction are his roommates. It seems appropriate, then, that a sociometric task used in this setting would have as its criterion the choice of roommate. If each patient were asked to choose those men he would most like to live with and those with whom he would least like to live, the sociometric status of each patient on the ward could be easily determined. This method has all the advantages outlined by Schauer and, in addition, is relatively simple and affected very little by intellectual level. The predictions of the present writer would then be as follows: In comparison with well-adjusted patients, poorly adjusted patients will show: a lower sociometric status, fewer mutual (reciprocated) choices, and less tendency to join voluntary ward groups.

[1] From the Veterans Administration Hospital, Oteen, North Carolina.

[2] This paper is abstracted, in part, from a Master's thesis completed at the University of North Carolina in January, 1959.

[3] Present address: Franklin D. Roosevelt Veterans Administration Hospital, Montrose, New York.

Methods

The data for this investigation were collected at the Oteen, North Carolina, Veterans Administration Tuberculosis Hospital.[4] The subjects used were the entire patient populations of three treatment wards. The total number was 160, but not all of these were interviewed. Some were too ill to participate, some were away on leave at the time of the investigation, while several refused to take part in the study. The total number interviewed was 128.

The majority of subjects were photographed on the ward and their pictures were taped to one side of a 3- by 5-inch index card. On the other side was the patient's name, while on both sides a code number appeared, which helped facilitate reference in the analysis of data and assured anonymity of the patient when desired. For those patients who were not present when the photographs were taken or who refused to be photographed, the same card was included without the picture. Two prints were made from each negative, the second being given to the patient. These pictures were a source of much excitement on the wards and contributed a great deal to the building of patient-interviewer rapport before any interviews were actually attempted.

At the beginning of each interview, the patient was told something about the nature of the study. He was assured that his answers would be held in the strictest confidence and was shown a number appearing at the top of the interview sheet in place of his name. Each patient was then presented with a stack of cards representing every patient on the ward, himself excluded. To save time and reduce the number of cards to be considered, each subject was first asked to pick out the pictures of those patients whom he knew. It was emphasized that the criteria for "knowing" must be more than merely recognizing a face. In most cases, the ability to name the patient in the photograph was sufficient. However, there were numerous instances of a subject's not knowing another by name but still being well enough acquainted to make judgments about him. In these cases the subject was asked: "Do you ever talk with him?" or "Do you know anything about him?" Most of the patients had little trouble differentiating people with whom they had at some time interacted from those merely recognized as being on the ward.

Second, the patient was asked: "Suppose you could pick anyone on the ward to have as a roommate. Which men out of these you know would you most like to live with?" The patient was allowed to make as many choices as he wanted, but was encouraged to choose at least five others. He was then asked to pick in the same manner those men he would least like to live with. Most patients expressed a much greater hesitancy to make this latter choice, but an attempt was made to get at least five rejections from each.

It was decided that staff ratings would be used as the criterion of patient adjustment. The major drawback to this procedure was that a particular staff member might have a unique or atypical view of what he thought constitutes a well-adjusted patient. To compensate for this, it was decided to use the ratings of only the professional staff members and to include several from each ward. The total number of staff members interviewed in this investigation was eleven. This sample consisted of the head physician and head nurse from each of the three wards, plus two additional nurses from two of the wards and one from the other.

When interviewing the staff members, the same picture cards were used, and since the subjects were well acquainted with all of the patients on their wards, it was not necessary to determine the number known. Each subject was asked to pick out the pictures of the 10 patients he thought had made the best adjustment to hospitalization and of the 10 who had made the least adequate adjustment. No attempt was made to define adjustment, and care was taken to make sure that the two groups were put in rank-order. For the five best and five most poorly adjusted patients, the staff members were questioned as to the criteria they used in ranking each of these.

After completing the staff interviews, it was necessary to select the final groups of well and poorly adjusted patients to be used in making comparisons on the sociometric variables. In order to do this, two factors had to be taken into account. First, how many staff members on a ward chose a particular patient as being well or poorly adjusted; and, second, where did he rank this patient in relation to others chosen in the same group? It was decided that the number of times chosen should be supplemented by a point system depending upon rank position. The following method was selected: Ranks 1, 2, and 3 were given three points; ranks 4, 5, 6, and 7 were given two points; ranks 8, 9, and 10 were given one point; not mentioned were given no points. In this manner, both rank-order and number of mentions could be taken into account by putting emphasis only on the general location of the ranks (top, bottom, middle). This ranking was performed on both patient categories, the 10 patients on each ward with the highest point totals being included in the two groups. Thus, the total number for each of these groups was 30. If there was any inter-staff disagreement concerning whether a patient was well or poorly adjusted, that patient was not included in either group.

As a final procedure, it was necessary to determine the criteria used by staff members in making adjustment ratings. It is possible that the main variable (social behavior) was frequently used as a criterion so as to automatically result in a substantial relationship in the predicted direction. As it turned out, however, the primary criteria for

[4] This study is only part of a larger investigation concerned with adjustment to hospitalization for tuberculosis. Henceforth, only those methods pertaining to this particular paper will be cited.

good adjustment concerned cooperation and ability to accept and understand the disease, while the main criteria for poor adjustment were inability to accept hospitalization, constant complaining and—most important—that the patient had a history of premature discharge. Thus, the poorly adjusted group comprised a large number of patients who had already shown their inability to adjust to hospitalization.

Results

A nonparametric sum of ranks test, as derived by Wilcoxon (12) and described by Walker and Lev (13), was used to evaluate the data from the individual wards. The Z score as seen in tables 1 and 2 indicates the probability that the two samples have been drawn from the same population. The crude totals as seen in these two tables do not directly enter into the computation of standard scores. These group scores help give a better idea of the magnitude of differences between the well and poorly adjusted patients.

It may be seen in table 1 that, in general, the poorly adjusted patients received fewer choices for roommate preference. These differences were striking on two of the wards and in the expected direction on the third. A chi-square median test (14) was used to evaluate the totals over all the wards, the result being highly significant.

The number of mutual choices entered into by the two groups of patients is illustrated in table 2. A mutual choice occurs when someone is preferred by a patient he himself has chosen. Again, these differences are marked in the cases of wards B and C, but are not significant for ward A. On all the wards the tendency is in the expected direction. Again, using the chi-square median test, the totals for all three wards are highly significant.

TABLE 1

Number of Positive Choices Received by Groups of Equal Size of Well and Poorly Adjusted Patients

Ward	Well Adjusted Patients	Poorly Adjusted Patients	Z	χ^2
A	62	53	0.76	—
B	115	38	2.87*	—
C	85	43	2.53*	—
Total	262	174	—	13.07*

* Indicates significance at the 0.05 level. Sum of ranks test used for ward comparisons, chi-square median test used for totals. Degrees of freedom = 1.

TABLE 2

Number of Mutual Choices Entered into by Groups of Equal Size of Well and Poorly Adjusted Patients

Ward	Well Adjusted Patients	Poorly Adjusted Patients	Z	χ^2
A	34	21	1.40	—
B	50	13	3.14*	—
C	34	13	2.23*	—
Total	118	47	—	13.07*

* Indicates significance at the 0.05 level. Sum of ranks test used for ward comparisons, chi-square median test used for totals. Degrees of freedom = 1.

TABLE 3

Total Number of Three- and Four-Man Voluntary Groups (Mutual Choices), Number of Group Members, and Number of Poorly Adjusted Group Members

Ward	Number of Groups	Number of Members	Number of Poorly Adjusted Members
Four-Man Groups			
A	0	0	0
B	1	4	0
C	2	8	1
Total	3	12	1
Three-Man Groups			
A	8	15	4
B	12	20	3
C	5	10	1
Total	25	45	8

In table 3 the total number of voluntary groups (groups of three or four mutual choices) is plotted. These are called "voluntary" due to the fact that they may include patients other than members of the room group to which a patient is arbitrarily assigned. No significance tests were attempted due to the fact that the poorly adjusted group was not identical with the group of 30 being used in other comparisons. All patients given a rating of poor adjustment were included here, this being approximately 30 per cent of the total number of patients on each ward. It is interesting to see that only one of 12 patients comprising three four-man groups received a poor adjustment rating. And of the 45

patients comprising 25 three-man groups, only 8 were rated as poorly adjusted. A fact of further interest results when the mean length of stay in the hospital for those 9 poorly adjusted patients who entered into voluntary groups is compared with that of the original group of 30 poorly adjusted patients:

Mean length of hospital stay:
9 patients—232.0 days
Mean length of hospital stay:
30 patients—183.2 days

This difference is of a magnitude which suggests that poorly adjusted patients will enter into voluntary groups on the wards, but that they do this at a much slower rate than do the well adjusted patients. Since it has been shown that the poorly adjusted patient has spent relatively fewer days in the hospital (4, 5, 15), it is possible that he will resort to premature discharge before he has had a chance to become integrated into the social structure of the ward.

Discussion

In general, the results of this study have shown relationships in the predicted direction. However, there were several instances of differences between the three wards. This finding is in substantial agreement with the results obtained by Shelton and Phillips (16), and again points to the importance of considering the hospital and even the ward as significant variables when evaluating research results.

Ward *A* had probably achieved the best social integration of the three at the time of the investigation. The patients on this ward had already established their social ties, the main social units being located in the room groups and the voluntary groups that became established between the various rooms. Among the poorly adjusted patients on this ward was a group of 4 who had been in the hospital somewhat longer than the rest. Their relatively longer period of residence and their ability to become accepted within the room group (if not the ward) undoubtedly decreased the choice differences between the well and poorly adjusted groups on ward *A*. Three of these 4 patients received premature discharges before the study was completed.

Ward *B* was nearly the opposite of ward *A*. Ward *B* was a relatively new one, many of the patients having been in residence less than two months. There were no clear-cut centers of social action. Whereas the small groups appeared to be the social units on ward *A*, all of ward *B* must be regarded as being in this situation. There were many mutual choices scattered over the entire ward, and the total number of choices was much greater in proportion to the number of rejections. This was, perhaps, partially a function of the newness of the ward and the uncertainty of how one was to respond to others. In other words, when in doubt, the subjects chose to say something positive.

A racial variable seemed to operate on ward *C*. Although it had the greatest population, there were fewer mutual choices than on either of the other two wards. This effect seemed to be due to ward administrative policies, for racial integration had not been completely carried out on ward *C* at the time of this study. Nearly all of the choices and rejections made by white patients were on the basis of race. The few mutual choices suggest that these were not always realistic, that is, choosing someone because of his race rather than for any favorable qualities he might possess.

Realizing the importance of these social psychologic variables, certain steps can be suggested which might help maximize patient adjustment and reduce premature discharge rates. Grouping patients by sociometric choice would seem advisable, especially in the case of the premature discharge recidivist, when one might make better use of the few adequate social contacts he has. Also, since a great many premature discharges are reactions to specific hospital situations, interpersonal or otherwise, it might be advisable to remove the patient from the stressful situation before he is actually forced to run away. This method of using an "irregular discharge ward" has been suggested by Lorenz and associates (17).

The need for more research in this area must also be stressed. It no longer seems feasible, at least with instruments presently in use, to try to isolate a unique tuberculous personality or attempt to differentiate adjustment groups by means of standard personality tests. Rather, there should be further efforts to create new tests and new scales for this purpose (1, 18). The poorly adjusted patient should no longer be thought of as psychopathic or neurotic but, rather, should be considered in terms of relationships between his particular need-system and environment.

And finally, much research needs to be directed toward the relationships between patients and

staff, as well as among the patients themselves. The methods used by Golann (19) show promise of throwing new light on the problem of patient-staff interaction. What types of social disturbance are most likely to result in premature discharge? How can one predict, at an early period, those patients who will fail to adjust socially? What type of ward social structure is most conducive to good adjustment? There has been little work done in this area of tuberculosis management, although it has often been pointed out as a fruitful field for the application of research findings.

Even in clarifying some of these relationships however, it is evident that poor adjustment and premature discharge are the result of a combination of variables rather than due to any single factor, be it personality, social adjustment, or the disease itself. One can see examples of patients who are so crippled in their interpersonal behavior that they seem to hold little hope of being able to adjust to any situation, but somehow manage to adjust to the hospital environment. Other patients, who may be more socially adept, are never able to come to terms with their disease or the hospital situation, and resort to premature discharge in an attempt to remove themselves from an unbearable environment of sickness, dependency, and restriction. Although an attempt has not been made to show that lack of social adjustment is the only cause of premature discharge, this study has brought to light some of the more important and seldom investigated variables that operate throughout the course of long-term hospitalization.

Summary

An investigation was made of the relationship between adjustment to long-term hospitalization for tuberculosis and adequacy of interpersonal relationships within the hospital. The subjects were the patient populations of three treatment wards. A correlation was predicted between hospital adjustment and adequacy in social relationships as measured by sociometric tests. The predictions were: In comparison with well adjusted patients, poorly adjusted patients will show: a lower sociometric status, fewer mutual choices, and less tendency to join voluntary ward groups.

Each subject interviewed was asked to choose those patients he would like most and those he would like least as roommates. At least three professional staff members were interviewed on each ward and were asked to pick the 10 patients who had made the best adjustment to hospitalization and the 10 who had made the least adequate adjustment of those on their wards. From these staff ratings, two groups of patients representing the extremes of adjustment were selected—10 well and 10 poorly adjusted patients from each ward. Thus, there was a total of 30 patients in each category. These two groups were then compared in terms of the three predictions, the first two of which were evaluated statistically. Comparisons were made on both a ward and a total-sample basis.

All of the results were in the expected direction and, in most cases, reached the 0.05 level of significance. The totals were highly significant, but substantial differences were observed between the wards themselves. These differences were discussed in the light of ward social structure and situational, rather than personal, variables. Suggestions were made concerning the use of these data toward promoting adequate patient adjustment and a reformulation of research objectives in the area.

Sumario

La Socialización de las Salas y el Ajuste al Hospital de Tuberculosis

Esta investigación versó sobre la relación entre el ajuste a la hospitalización a largo plazo por tuberculosis y la suficiencia de las relaciones personales dentro del hospital. Los sujetos fueron los enfermos alojados en tres salas de tratamiento. Se anticipaba una correlación entre el ajuste al hospital y la suficiencia de las relaciones sociales, medidas éstas con pruebas sociométricas. Lo predicho fué: comparados con los enfermos bien ajustados, los mal ajustados revelarán: inferior estado sociométrico, menos selecciones mutuas, y menor tendencia a ingresar en los grupos voluntarios de las salas.

A cada sujeto entrevistado se le pidió que escogiera a los enfermos que más le gustarían y a los que menos le gustarían como compañeros de cuarto. Se entrevistó a lo menos a tres miembros del claustro profesional en cada sala, pidiendóseles que escogieran en la misma a los 10 enfermos que habían mostrado el mejor ajuste a la hospitalización y a los 10 que habían hecho el peor ajuste. A base de esta clasificación por el claustro, se formaron dos grupos de enfermos que representaban los extremos del ajuste: 10 enfermos bien ajustados

y 10 mal ajustados de cada sala. Había, pues, así un total de 30 enfermos en cada categoría. Se compararon entonces estos dos grupos con respecto a las tres predicciones, valorándose estadísticamente las dos primeras de éstas. Las comparaciones se hicieron a base de salas y de la muestra total.

Todos los resultados tomaron la forma esperda y, en la mayoría de los casos, no se desviaron más de 0.05 en su exactitud. Los totales fueron sumamente significativos, aunque se notaron diferencias substanciales en las salas mismas. Se discuten estas diferencias a la luz de la estructura social de las salas y los factores variables en la situación más bien que en las personas mismas. Se ofrecen sugestiones relativas al empleo de estos datos con mira a fomentar el ajuste adecuado de los enfermos y a la reformulación de objetivos de investigación en la zona abarcada.

Resume

Vie sociale de salle et adaptation à l'hopital pour tuberculeux

L'auteur a fait des recherches sur la relation entre l'adaptation à une longue hospitalisation pour tuberculose et les rapports entre individus à l'intérieur de l'hopital. Les sujets étudiés furent pris parmi les malades de trois salles de traitement. On avait prévu une corrélation entre l'adaptation à l'hopital et les rapports sociaux jugés adéquats selon les réactions sociomètriques. Les faits prévus furent: en les comparant avec des malades bien adaptés, les malades mal adaptés montreront: un niveau sociomètrique plus bas, des choix mutuels plus rares, et moins de tendances à joindre librement des groupes dans la salle.

A chaque sujet interrogé, on demanda de choisir les malades qu'il prèfèrerait et quels malades il aimerait le moins comme compagnons de chambre. Dans chaque salle, on interrogea au moins trois membres de l'état-major médical et on leur demanda de choisir les 10 malades s'étant le mieux adapté à l'hospitalisation et les 10 malades s'étant le moins bien adapté dans la salle. On eut ainsi a partir de ces groupes donnés, deux groupes de malades représentant les extrêmes d'adaptation furent choisis: 10 bien et 10 mal adaptés pour chaque salle. On eut ainsi un total de 30 malades dans chaque catégorie. On compara ces deux groupes en suivant ce qui avait été prévu les deux premiers furent évalués au point de vue statistique. Les comparaisons furent faites à la fois par salle et par échantillon.

Tous les résultats furent confirmés comme il avait été prévu et, dans la plupart des cas, ils atteignirent un niveau de signification égal à 0.05. Les totaux furent très éloquents, mais des différences notables furent observées dans les salles elles-mêmes. Ces différences furent discutées en considérant plus la structure sociale et la situation que les variantes personnelles. On a proposé d'utiliser ces renseignements pour améliorer l'adaptation du malade d'une façon convenable et pour trouver d'autres buts de recherches dans ce domaine.

Acknowledgment

For their valuable assistance, the writer would like to thank Dr. John Thibaut of the University of North Carolina who directed the thesis; and Dr. S. E. Vineberg, former Chief, Psychology Service, Veterans Administration Hospital, Oteen, North Carolina, now at Vanderbilt University.

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Notes

SERIAL DETERMINATION OF SERUM GLYCOPROTEINS IN PATIENTS WITH PULMONARY TUBERCULOSIS

Elevated values for serum glycoprotein (serum polysaccharide or protein-bound carbohydrate) were reported in patients with tuberculosis by Lustig and Langer,[1] and were studied in more detail by Seibert and associates.[2] These results subsequently have been confirmed by a number of investigators.[3-5] Elevated values for serum glycoprotein and seromucoid have also been reported in guinea pigs following tuberculosis produced experimentally.[6] As the serum glycoprotein determination has been useful as an aid in evaluating the status of patients with rheumatoid arthritis,[7, 8] rheumatic fever,[9] and cancer,[10] studies of the changes of the serum glycoproteins in tuberculous patients under therapy should be of interest. Lawrence and co-workers[11] found elevation of serum glycoproteins and seromucoid in patients with tuberculosis. The values frequently were normal if the patient had had antituberculous chemotherapy prior to admission to the hospital. The chemical values correlated with the hospital course. The patients with retrogressive disease showed elevations on admission and declines toward normal of the seromucoid, total glycoprotein, gamma globulin, and gamma-globulin polysaccharide. The patients with more chronic infections exhibited little change in their glycoproteins during their hospitalization. Edward,[12] reporting seromucoid values only, found that this material was elevated in patients with active tuberculosis and declined toward normal with antituberculous chemotherapy. A rough correlation was found between the extent of disease and the seromucoid value, and a good correlation between this value and disappearance of tubercle bacilli from the sputum. Since these have been the only studies of the serial changes in glycoproteins in tuberculosis, further serial studies seemed warranted.

Total serum glycoprotein as protein-bound hexose was determined by the tryptophan method of Shetlar and associates.[13] The glycoprotein was also expressed as a percentage of total serum protein since this expression compensates for changes in serum protein. Seromucoid was isolated from serum by the method of Winzler and coworkers[14] and was quantitated by hexose determination by the tryptophan method. Total protein was determined by the biuret method as described by Weichselbaum.[15] Gamma globulin was precipitated by one-third-saturated ammonium sulfate according to the method of Jager and Nickerson.[16] Hexose was determined in this precipitate by the tryptophan method,[13] and gamma-globulin protein was determined by the biuret reaction.[15] The ratio of gamma-globulin glycoprotein to gamma globulin was also calculated. Fractionation of albumin and pseudoglobulin was made by the Milne method,[17] and the hexose content of these fractions was determined by the tryptophan reaction. Erythrocyte sedimentation rates were determined by the Wintrobe method.

Forty-three male patients with active pulmonary tuberculosis were studied at the Oklahoma City Veterans Hospital. Blood specimens were obtained every two weeks, the first specimen being taken at the time chemotherapy was initiated. Twenty-seven of these 43 patients were new cases with no previous drug therapy and 16 of

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TABLE 1

COMPARISON OF TOTAL SERUM GLYCOPROTEIN/PROTEIN RATIO FOR TUBERCULOUS PATIENTS WITH CONTROL GROUP FOR FIRST MONTH OF STUDY

Period	Month	Number in Group	Total Glycoprotein	Glycoprotein-Protein Ratio	Gamma-Globulin Glycoprotein	Gamma-Globulin Glycoprotein-Protein Ratio	Seromucoid
			mg./100 ml.	*per cent*	*mg./100 ml.*	*per cent*	*mg./100 ml.*
1	1	43	168 ± 5.3	2.3 ± 0.06	30.8 ± 1.7	2.3 ± 0.06	19.3 ± 1.0
2	2 and 3	31	151 ± 3.6	2.1 ± 0.05	27.0 ± 1.6	2.3 ± 0.08	14.7 ± 0.9
3	4 and 5	16	147 ± 7.7	2.1 ± 0.17	29.2 ± 1.0	2.5 ± 0.08	14.7 ± 0.8
4	6 and 7	9	149 ± 5.4	2.0 ± 0.08	26.2 ± 1.4	2.1 ± 0.08	12.4 ± 1.0
5	8 and 9	5	141 ± 12.6	1.9 ± 0.05	23.0 ± 2.0	2.6 ± 0.18	12.0 ± 1.3
6	10 and 11	6	159 ± 10.1	2.2 ± 0.07	25.0 ± 0.6	2.5 ± 0.15	16.3 ± 1.1
	Normal subjects	28	121 ± 1.7	1.7 ± 0.02	21.0 ± 0.7	2.3 ± 0.05	12.0 ± 0.8*

All values are expressed as mean ± the standard error.
* The normal for seromucoid is based on 11 subjects.

the patients had had some drug therapy prior to their inclusion in the study.

Tuberculous chemotherapy was directed by the ward physicians and was not altered by this study. The new patients were treated according to the protocols established by the Veterans Administration Armed Forces Cooperative Studies.[18]

Before evaluation of the chemical data, all charts and roentgenograms were reviewed, and the following information was recorded for each patient: activity, stage of disease (utilizing National Tuberculosis Association (NTA) standards[19]), presence and size of cavities, per cent of lung tissue involved estimated from the chest films, previous chemotherapy, presence of fever at admission, presence of tubercle bacilli in the sputum, and improvement for each period. The estimate of the per cent of lung tissue involved by the tuberculous process was made to provide a better means of comparing the effect of the amount of inflammatory tissue present. National Tuberculosis Association classification utilizes cavity size as well. To facilitate handling of the data, an average was obtained of all values during the first thirty days for each patient. Similar averages were calculated for all values obtained during each succeeding sixty-day period. The control group consisted of 28 apparently healthy subjects for all fractions except the seromucoid, which included 11 subjects.

Results: The total serum glycoprotein/protein ratio during the first month of study for the patients with tuberculosis was 2.33 ± 0.06 as compared with 1.71 ± 0.02 for the control group (table 1). This difference was significant ($t = 9.85$, $P < 0.01$). When the patients were divided according to the extent of their tuberculosis by NTA standards, the total serum glycoprotein percentage during the first month of observation correlated with the severity of the disease (figure 1). The difference between the groups with minimal and with far advanced disease gave a P value of <0.05 ($t = 2.41$). The total serum glycoprotein percentage also correlated with the estimate of the percentage of lung involved (<25 per cent lung involved = 2.16 ± 0.1, 25–50 per cent involved = 2.31 ± 0.1 and >50 per cent involved = 2.52 ± 0.2). The differences are all significant ($P < 0.05$). During the first month those patients whose sputum became negative for tubercle bacilli had a mean serum glycoprotein percentage of 2.06 ± 0.0, while the average value for those with tubercle bacilli in the sputum was 2.35 ± 0.09 per cent. This difference was not significant ($t = 1.91$, $P > 0.05$). The presence of cavity, fever, and prior therapy did not influence the value. The erythrocyte sedimentation rate correlated significantly with the total serum glycoprotein percentage ($r = 0.48$, $P < 0.01$). Comparison between the group of patients whose chest films showed early improvement with those whose films did not, showed no significant difference ($t = 1.33$, $P > 0.1$). The total glycoprotein values yielded entirely similar results except that the initial values of the patients whose sputum became noninfectious were significantly lower than of those who continued to discharge tubercle bacilli.

The seromucoid was significantly elevated to 19.3 ± 1.0 mg. per 100 ml. compared with a normal control figure of 12.0 ± 0.8 mg. per 100 ml. ($t = 5.62$, $P < 0.01$). The extent of the tuberculosis did not appear to affect the seromucoid level. There was no significant difference in the initial

[18] Transactions of the Thirteenth and Fourteenth Conferences on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1954 and 1955.

[19] Diagnostic Standards and Classification of Tuberculosis, National Tuberculosis Association, 1955.

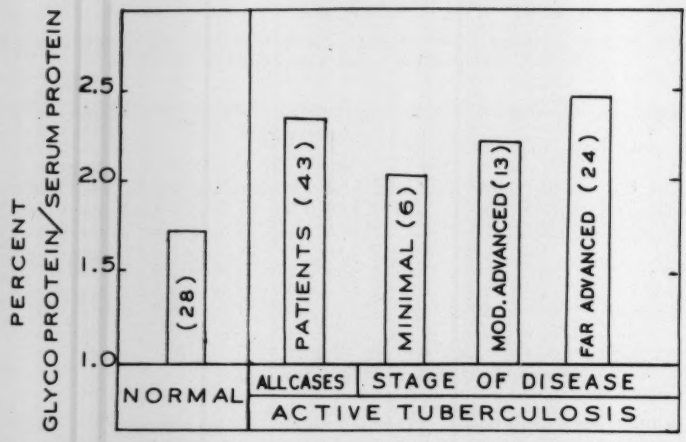

FIG. 1. Mean total serum glycoprotein percentage during the first month of observation of 43 hospitalized tuberculous patients who received chemotherapy. The figure in parentheses on each bar shows the number of persons for each group.

seromucoid levels between the group of patients whose disease improved by roentgenographic examination and those whose disease remained the same ($t = 0.86$, $P > 0.3$). The erythrocyte sedimentation rate did not correlate with initial values of seromucoid ($r = 0.21$, $P > 0.05$), and the presence of cavity, fever, and prior therapy did not influence the amount of seromucoid.

The mean gamma-globulin glycoprotein during the initial period was 30.8 ± 1.7 mg. per 100 ml. as compared with a control of 21.0 ± 0.7 mg. per 100 ml. This difference was significant ($t = 4.26$, $P < 0.01$). When these data were analyzed for the various factors, the only significant difference was in the mean gamma-globulin glycoprotein value for the patients with less than 25 per cent lung involved as compared with more than 50 per cent (27.7 mg. and 34.1 mg., $t = 1.88$, $P < 0.05$). There was no correlation with the sedimentation rate ($r = 0.29$, $P > 0.05$). There was no significant difference in the elevation of the initial gamma-globulin glycoprotein values in the patients who showed improvement and those who did not ($t = 0.298$, $P > 0.8$).

The gamma-globulin glycoprotein during the first month of observation expressed as a percentage of gamma globulin did not differ significantly from the controls (2.30 ± 0.06 per cent and 2.31 ± 0.05 per cent).

The mean values obtained for the glycoprotein fractions declined toward normal during subsequent observation periods. The amount of change for each clinical category and glycoprotein fraction between each period were subjected to statistical comparisons and the results of these comparisons are summarized in table 2, which shows the "t" value of each comparison made between the *change* of chemical values for each clinical category, and indicates which changes were significant at the 95 per cent confidence level or higher.

For the whole group of patients with active tuberculosis, each of the serum glycoprotein substances measured decreased during the second and third months of observation from the average values found during the first month. This decline was significant for each of the three substances measured: total serum glycoprotein ($t = 5.76$, $P < 0.01$), seromucoid ($t = 3.78$, $P < 0.01$), and gamma-globulin glycoprotein ($t = 3.6$, $P < 0.01$). In addition, the total glycoprotein/protein ratio decline was significant ($t = 4.83$, $P < 0.01$). However, the gamma-globulin glycoprotein/gamma globulin ratio did not decline significantly ($t = 0.44$, $P > 0.50$). During subsequent months of observation the total serum glycoprotein showed no further significant decline. Following the initial drop, the seromucoid fraction remained stable within the normal range during the second through the fifth months, but during the sixth and seventh months the mean value was significantly lower than during the preceding periods ($t = 3.24$, $P < 0.02$). The glycoprotein fraction of the gamma globulin behaved similarly, remaining near the upper limit of the normal range during the second through the fifth months, then declining significantly into the normal range during the sixth and seventh months ($t = 5.55$, $P < 0.01$)

TABLE 2

SUMMARY OF STATISTICAL COMPARISON

"t" Values Change in Mean Value between Periods of Observation

Comparison	d/f	Total Glycoprotein	Glycoprotein-Protein Ratio	Seromucoid	Gamma-Globulin Glycoprotein	Gamma-Globulin-Glycoprotein-Protein Ratio
Active tuberculosis						
Change period 1 to period 2	30	5.76*	4.83*	3.78*	3.60*	0.44
Change period 2 to period 3	14	1.59	1.64	0.70	0.85	2.43†
Change period 3 to period 4	6	0.56	0.87	3.24†	5.55	0.01
Improving roentgenographically						
Change period 1 to period 2	13	3.76*	2.82*	2.24†	3.13*	2.73†
Change period 2 to period 3	7	1.69	1.32	1.01	0.34	1.78
Change period 3 to period 4	3	0.29	0.56	1.33	3.84	1.31
Same roentgenographically						
Change period 1 to period 2	12	3.70*	2.23†	2.28†	3.18*	0.571
Change period 2 to period 3	5	0.34	0.07	1.07	1.64	1.58
Change period 3 to period 4	1	0.65	2.02	<0.65	1.96	1.04
Improving roentgenographically vs. Same roentgenographically						
Change period 1 to period 2	26	0.32	1.23	0.27	1.72	1.50
Change period 2 to period 3	13	0.55	0.50	0.17	0.83	0.52
Change period 3 to period 4	6	0.08	0.16	<0.08	<0.08	0.76
Sputum became negative for tubercle bacilli						
Change period 1 to period 2	13	3.20*	3.87*	2.66†	1.50	0.39
Change period 2 to period 3	9	0.91	0.13	0.65	0.13	2.89
Change period 3 to period 4	5	<1.40	<1.40	2.31	7.93*	1.40
Sputum remained positive for tubercle bacilli						
Change period 1 to period 2	12	5.02†	2.71*	2.22†	1.91	0.20
Change period 2 to period 3	2	3.32	2.43	0.00	1.14	0.16
Sputum negative vs. positive for tubercle bacilli						
Change period 1 to period 2	26	3.12	1.91	0.41	0.69	1.44
Change period 2 to period 3	10	1.27	0.44	0.44	0.89	0.93

d/f Degrees of freedom.
* $P < 0.01$.
† $P < 0.05$.

The gamma-globulin glycoprotein/gamma-globulin ratio did not decline, but showed a significant increase during the fourth and fifth months ($t = 2.43$, $P < 0.05$). However, no other significant changes occurred in this ratio. The rises noted during the tenth and eleventh months may be attributed to the influence of a remnant of patients who had progressive disease.

The group of patients with roentgenographic improvement showed a significant decline of all these substances and for the total glycoprotein expressed as per cent. However, the group of patients whose roentgenograms remained the same also showed a significant decline in the concentration of each of these substances. Comparison between these two groups of the amount of change in mean concentration of these substances from the first month to the second and third months showed no significant difference.

Only 3 of the patients with active tuberculosis showed progressive disease by roentgenographic examination during the second and third months as compared with the first month of observation. Analysis of the glycoprotein determination on this small group showed a significant decrease in the total serum glycoprotein ($t = 6.65$, $P < 0.05$).

However, none of the other substances showed a significant change. A comparison was made of the amount of change that occurred in concentration of each substance from the first month to the second and third months between the patients who improved or remained the same with those who had progressive disease. A significant difference was found in the decline of the total serum glycoprotein concentration between the patients who improved and those who had progressive disease ($t = 2.81$, $P < 0.02$), but the variation occurring in the patients whose disease was unchanged did not differ significantly from that in the patients who had progressive disease ($t = 2.09$, $P > 0.05$). However, the total serum glycoprotein percentage of the total serum protein showed significant differences in rate of change during this period, both for the progressive versus the improving groups ($t = 3.11$, $P < 0.01$) and for the progressive versus the unchanged groups ($t = 3.65$, $P < 0.01$). The glycoprotein decreased less in the progressive group. These patient groups showed no significant changes in the other substances.

Grouping the patients as to whether their sputum became negative or remained positive for tubercle bacilli and analyzing the changes in the glycoprotein fraction concentrations from the first month to the second and third months showed significant decreases in the total serum glycoprotein, the total glycoprotein as percentage of serum protein, and the seromucoid for both groups and no difference in the two categories. The gamma-globulin glycoprotein concentration, and this substance expressed as a percentage of gamma globulin, did not change significantly.

* * *

Serum glycoprotein fraction elevations have been reported in a number of different pathologic processes such as infection, arthritis, and cancer.[20] Apparently the increase of these substances is a general body-response to inflammation. It is not surprising to discover that the concentration of these substances returns toward normal values with the subsidence of the inflammatory process which caused their elevation.

In this series of 43 cases of active tuberculosis there is good correlation between the elevation of the serum glycoprotein values and the criteria used for evaluation of the extent of activity of the disease. As noted in the groups of patients with and without roentgenographic evidence of improvement and in those whose sputum became negative or remained positive for tubercle bacilli, there is a significant decrease of the glycoprotein concentrations. However, these substances declined similarly in patients who showed improvement in their roentgenographic status and in those who remained the same and independent of sputum conversion. This lack of significant difference possibly indicates that the inflammatory tissue processes of pulmonary tuberculosis are subsiding in both groups, that the roentgenographic evidence of structural improvement occurs more slowly, and that infection per se is not responsible for the elevations noted. These findings support Shetlar's suggestion that elevation of the glycoprotein fractions correlates with the extent of tissue injury and with tissue proliferation.[8, 20]

These results are in essential agreement with the reports of Lawrence and associates,[11] Edward[12] and Galletti and co-workers.[21] Certain differences, however, should be noted. Lawrence,[11] for example, found that patients with prior therapy and a more chronic form of the disease had normal values. The values for this group in the present series were not different from the values in those who were new treatment cases. Both Lawrence and Edward observed that the seromucoid levels correlated with the extent of the disease. The patients in the present study did not exhibit this correlation as far as the seromucoid levels were concerned, although the other factors did correlate. The reason for this difference is not evident. Edward found that roentgenographic improvement and reversal of infectiousness were reflected by lowering of values, whereas, in the present study, reduction in values was noted whether or not there was roentgenographic change and whether or not the sputum remained positive for tubercle bacilli. Again, there is no satisfactory explanation for this apparent discrepancy.

Since the glycoprotein values are related to the amount of activity of the pulmonary tuberculosis, serial determination of these substances apparently provides a means of evaluating a patient's response to therapy. An example of this application is seen in the last period of observation (*see* table 1). The mean values shown in the last period of observation represent a small number of patients who had left the hospital earlier against advice and returned at this time, with an exacerbation of their tuberculosis. As mentioned earlier, the small group with progressive disease exhibited a reduced rate of return to normal. This was also observed by Lawrence and associates.[11] It seems probable that the serum glycoproteins represent another parameter which is independent of many

[20] SHETLAR, M. R.: Texas Rep. Biol. & Med., 1952, *10*, 228.

[21] GALLETTI, F., *et al.*: Acta tuberc. Scandinav., 1955, *31*, 185.

of the factors ordinarily used to determine improvement of the tuberculous process.

In summary: Serial determinations of several serum glycoprotein fractions have been made in 43 male patients undergoing chemotherapy for active pulmonary tuberculosis. Initially, the values of the total serum glycoprotein, serum glycoprotein/protein ratio, the seromucoid, and the gamma-globulin glycoprotein were elevated to a significant degree above normal. These values, with the exception of the seromucoid, seem to be proportional to the extent of the inflammatory response. The presence of cavity, fever, previous treatment, reversal of infectiousness, or roentgenographic improvement did not influence the initial values. The total serum glycoprotein, seromucoid, and gamma-globulin glycoprotein values decreased during antimicrobial therapy. The small group of patients with progressive disease showed less tendency to return toward normal. Determination of these substances provides an additional means to evaluate the response to therapy.

HAROLD G. MUCHMORE
JANE A. FAULKNER
JAMES F. HAMMARSTEN
FRANCES G. FELTON
GEORGE WINKELMAN
Medical Service and Clinical Laboratory Service
Veterans Administration Hospital; and
Departments of Microbiology and Medicine
University of Oklahoma
Oklahoma City, Oklahoma

July 13, 1959

A NEW ANTITUBERCULOUS AMINO ACID (α-HYDROXY-γ-OXO-L-NORVALINE)

An antituberculous amino acid has been isolated from the culture broth of *Streptomyces Akiyoshiensis nov. sp.*[1] This was identified as α-hydroxy-γ-oxo-L-norvaline, abbreviated as HON.[2] The compound was also prepared by chemical synthesis.[2]

Interestingly enough, this amino acid is effective only against strains of human and bovine tubercle bacilli and certain of the unclassified mycobacterial strains. All other bacteria tested so far, including avian tubercle bacilli and saprophytic mycobacteria, were found to be insusceptible to the acid.

The minimal inhibitory concentration of the amino acid *in vitro* varies widely according to the culture medium used. Against both human and bovine tubercle bacilli, the inhibitory concentration was 2 to 4 γ per ml. in "ammonium sulfate medium,"[3] 10 to 20 γ per ml. in Kirchner medium, and 200 to 400 γ per ml. in Dubos medium.

These discrepancies were accounted for by the antagonistic effect of amino acids (glutamine, asparagine, glutamate, and aspartate) and of ferrous compounds.

Although this antituberculous amino acid is more stable in phosphate buffer at pH 6 than at pH 8, the minimal inhibitory concentration against H37Rv was the same over a pH range of 5.5 to 8.5 in Kirchner medium.

Tubercle bacilli of the H37Rv strain, resistant to the amino acid, can be obtained by repeated transfers in Kirchner medium. After 25 such transfers, the bacilli gained 64 fold resistance, and their population was found to be homogeneous in character. On the other hand, the emergence of isoniazid-resistant H37Rv *in vitro* was markedly retarded as compared with the control series in the presence of 2 to 4 γ per ml. of the amino acid in the Kirchner medium.

The antituberculous effect of HON against intracellular tubercle bacilli was estimated in tissue culture of guinea pig monocytes essentially according to the Suter method.[4] The experimental results showed that 160 γ of HON per ml. were equivalent in effectiveness to 10 γ of streptomycin per ml.

As to the acute toxicity of the amino acid for mice, the LD_{50} was 5,200 mg. per kg. by the intravenous route, 8,000 mg. per kg. by the subcutaneous route, and 7,600 mg. per kg. by the oral route. Measurement of HON in the blood can be made colorimetrically by the use of triphenyltetrazolium chloride after deproteinization by sodium tungstate. When 100 mg. of HON per kg. were administered to a rabbit subcutaneously, a peak concentration of 177 γ per ml. was attained after one hour and fell to 4 γ per ml. after five hours. By the oral route, a peak of 120 γ per ml. was obtained after two hours, but a concentration of 73 γ per ml. was still present after five hours.

The therapeutic effect upon experimental tuberculosis in mice was tested by a routine "survival test" using the H37Rv strain.[5] From comparison with the ST_{50}, a therapeutic effect corresponding to the subcutaneous administration of 0.2 mg. of streptomycin per day can be obtained by the administration of 1.0 to 2.0 mg. of HON per day either subcutaneously or by mouth.

It is interesting to observe that similarly infected mice, fed with a diet containing 0.05 per cent of HON, enjoyed a better therapeutic effect than those given a diet with 0.25 per cent of PAS.

A preliminary experiment with guinea pigs has shown that 100 mg. of the amino acid per day (from the second through the eighth weeks) exerted a therapeutic effect comparable to 5 mg. of streptomycin per day.

A synergistic effect has been noted by combining HON with zygomycin A (a new paromomycin-like antimicrobial) both *in vitro* and *in vivo*.

In view of the specific effectiveness of HON upon pathogenic mycobacteria, it is suggested that this amino acid might also affect *M. leprae* in humans. Further study of this amino acid seems worth while, and investigations are now being continued.

KEN-ICHI KANAZAWA
KANJI TSUCHIYA
TOYOSHIGE ARAKI
Research Laboratories
Takeda Pharmaceutical Industries, Ltd.
Osaka, Japan

December 28, 1959

[1] Descriptions of this strain will be published elsewhere by K. Nakazawa and his associates.

[2] MIYAKE, A.: (Chem. Pharm. Bull., in preparation)

[3]

$Na_2HPO_4 \cdot 12H_2O$	3.0
KH_2PO_4	4.0
$MgSO_4 \cdot 7H_2O$	0.6
Na citrate	2.5
Glycerol	20.0
$(NH_4)_2SO_4$	1.0
Distilled water	900.0
Bovine plasma Fraction V, added after autoclaving	100.0

In this medium, the minimal inhibitory concentration of streptomycin, PAS, and isoniazid against the H37Rv strain was estimated as 4 γ per ml., 1 γ per ml., and 0.1 γ per ml., respectively.

[4] SUTER, E.: J. Exper. Med., 1952, *96*, 137.

[5] ARAKI, T., *et al.*: Ann. Rep. Takeda Research Lab., 1956, *15*, 113.

OBSERVATIONS ON THE PIGMENT PRODUCTION OF UNCLASSIFIED MYCOBACTERIA UNDER CONDITIONS OF INCUBATION IN THE LIGHT AND IN THE DARK[1]

In the past, because of the pressure from the clinician for rapid culture identifications, it has been the practice of this laboratory to attempt to differentiate the unclassified mycobacteria by their pigment production in the presence and absence of light as soon as culture growth would permit. Unfortunately, this practice has led to a few premature reports to the clinicians which later had to be revised. It has also been observed upon several occasions that the pigments on which the classification was based were quite different from those pigments present on their respective drug-susceptibility controls when examined several weeks later. These two observations raised the question of just how long one should incubate cultures under light and in the dark before reliable observations could be obtained.

Runyon[2] divides the unclassified mycobacteria into three groups according to their pigment production, i.e.: photochromogens, nonphotochromogens, and scotochromogens. Whereas the photochromogens are yellow in the light and white-to-buff in the dark, the scotochromogens are red-orange in the light and yellow-to-pale-orange in the dark. All others, except rapid growers, are grouped as nonphotochromogens. This group includes those unclassified mycobacteria which produce the same pigment both under light and in the dark. In an attempt to further categorize the nonphotochromogens, which constitute the predominating group of the unclassified mycobacteria isolated in the Southeast, the nonphotochromogens have been divided into two groups: (*1*) those which are buff-to-ivory, and (*2*) those which produce the same degree of yellow-to-orange pigment in both the light and the dark. These are presently labeled nonphotochromogen III-A and nonphotochromogen III-B, respectively.

To answer the question concerning the length of incubation time and to evaluate the proposed grouping modification, a series of observations was made on several groups of unclassified mycobacteria which had been subjected to incubation in the presence and absence of light over a four-week period.

Five representative cultures from each of three groups, i.e., *Group III-A* nonphotochromogens (nonpigmented), *Group III-B* nonphotochromogens (pigmented), and *Group II* scotochromogens were selected from stock cultures for study (table 1). Suspensions of each culture were made in Dubos medium according to the technique of Dunbar and associates.[3] For each culture, five Löwenstein-Jensen medium slants were inoculated from the faintly turbid suspension, one of which was exposed to light, and the remaining four covered with dark paper. All were incubated at 37°C. A 36-inch, cool white, 30-watt, General Electric fluorescent bulb served as the light source. The cultures were 8 inches from the bulb. At this distance, the light intensity was 150 candles per square foot as measured with a Weson, Master No. 3, light meter.

TABLE 1

ORIGINAL CLASSIFICATION OF CULTURES TESTED

Culture Number	Original Classification
TAM 33	Group II scotochromogen
TAM 60	Group II scotochromogen
TAM 70	Group II scotochromogen
TAM 59	Group II scotochromogen
TAM 81	Group II scotochromogen
TAM 21	Group III-B nonphotochromogen (pigmented)
TAM 36	Group III-B nonphotochromogen (pigmented)
TAM 25	Group III-B nonphotochromogen (pigmented)
TAM 76	Group III-B nonphotochromogen (pigmented)
TAM 27	Group III-B nonphotochromogen (pigmented)
TAM 6	Group III-A nonphotochromogen (nonpigmented)
ORL 1	Group III-A nonphotochromogen (nonpigmented)
TAM 10	Group III-A nonphotochromogen (nonpigmented)
TAM 16	Group III-A nonphotochromogen (nonpigmented)
TAM 8	Group III-A nonphotochromogen (nonpigmented)

At the end of one week, the light culture and one of the corresponding dark cultures were examined, after which both were incubated in the presence of light. At the end of the second week

[1] This investigation was supported in part by the National Institute of Health Advisory Council on Allergy and Infectious Diseases, Grant 2377—"Pulmonary Disease and 'Atypical Acid-fast Bacilli'."

[2] RUNYON, E. H.: Am. Rev. Tuberc., 1955, *72*, 866.

[3] DUNBAR, F. P., *et al.*: Am. Rev. Tuberc., 1958, 77, 350.

TABLE 2

AMOUNT OF GROWTH AND PIGMENT PRODUCED OVER A FOUR-WEEK PERIOD

Culture Number	1st Week		2nd Week		3rd Week		4th Week	
	Light	Dark	Light	Dark	Light	Dark	Light	Dark
TAM 33	2+	3+	3+	3+	3+	3+	3+	4+
	y-yo	b	r-o	b-y	r-o	y	r-o	y
TAM 60	2+	4+	2+	3+	2+	4+	2+	4+
	y-yo	y-o	r-o	r-o	r-ro	yo-ro	r-o	yo-ro
TAM 70	1-2+	3+	2+	4+	3+	4+	2-3+	4+
	y-yo	y	o	y	y-o	y-o	y-o	y-o
TAM 59	1+	3+	1+	3+	2+	4+	2+	4+
	y	y	y-o	y-o	r-o	y-yo	r-o	y-yo
TAM 81	1+	2+	1+	3+	2+	4+	2+	4+
	y-yo	y-o	r-o	y	r-ro	y-o	r-ro	y-o
TAM 21	1+	2+	1+	3+	2+	3+	2+	3+
	y	b	y-o	b-y	y-o	t-y	y-yo	y
TAM 36	1+	2+	1+	3+	2+	3+	2+	3+
	y	b-y	y	y	y	y	y	y
TAM 25	2+	3+	3+	4+	3+	4+	3+	4+
	y	y	y	y	y	y	y	y
TAM 76	1+	3+	2+	3+	3+	4+	3+	4+
	y	y	y-o	y	r-o	y-yo	r-o	y-o
TAM 27	1+	2+	1+	3+	2+	3+	2+	3+
	l-y	b-y	y	b-y	y	b-y	y	y
TAM 6	2+	3+	3+	3+	3+	4+	3+	4+
	b-i	b-i	b-i	b-i	b-t	i-tc	b-t	b-c
ORL 1	1+	3+	2+	3+	3+	3+	2+	3+
	b	b-i	b-k	b-c	b-t	b-ct	b-t	b-c
TAM 10	1+	2+	2+	3+	2+	3+	3+	3+
	b-ly	b-i	y	b-y	y-yo	b-ty	by-yo	y-t
TAM 16	2+	3+	3+	3+	4+	4+	4+	4+
	b	b-i	b-i	b-i	i-c	b-i	b-i	i-c
TAM 8	2+	3+	3+	3+	4+	4+	4+	4+
	b-i	b-i	b-c	b-ic	c-t	b-c	b-t	b-c

1+—50–200 colonies
2+—innumerable discrete colonies
3+—less than confluent
4+—confluent

y—yellow
o—orange
b—buff
r—red
t—tan

l—lemon
i—ivory
c—cream
k—khaki

these cultures were re-examined along with another of the cultures incubated in the dark; then all were again re-exposed to light incubation. This was repeated throughout the four-week period.

Recorded in table 2 are the observations of the pigment development over the four-week period. It is readily apparent that a report of identification based on pigment production in the light and in the dark at the end of one week would be unreliable. For example, *TAM-33*, *TAM-21*, *TAM-10* and, conceivably, *TAM-36* and *TAM-27* could easily be mistaken for photochromogens of *Group I* if the conclusions were based on one-week observations alone.

At the end of the second week the color reaction of all cultures had become more accentuated, probably due to increased growth. This was more obvious in the third week, and the results of the fourth week were essentially the same as those of the third week. An experienced observer can draw fairly reliable conclusions from the results obtained at the end of the second week, although there still may be pitfalls, i.e., *TAM-33*, *TAM-21*, *TAM-27*, and *TAM-10*, which again may possibly be identified as photochromogens. Furthermore, *TAM-59* could be identified as a member of the nonphotochromogens of *Group III-B*.

As the incubation time increases, more reliability can be placed on the results obtained. At the end of the third week, a lay observer would have little trouble in interpreting his observations, although even here difficulties arise. It might be argued, and rightly so, that *TAM-70* belongs to the nonphotochromogens of *Group III-B* rather

than to those of the *Group II* scotochromogens, and that *TAM-76* belongs to *Group II* rather than to *Group III-B*. Here are cases of premature conclusions mentioned earlier. In addition, *TAM-10* might be considered a nonphotochromogen of *Group III-B* instead of a *Group III-A* nonphotochromogen.

Upon repeated light and dark incubation, an occasional culture originally classified as a nonphotochromogen of *Group III-B* has been observed to produce that pigment type associated with the *Group II* scotochromogen (and vice versa). Although it is not apparent here, this phenomenon has been encountered with the culture labeled *TAM-21*. Moreover, *TAM-70* and *TAM-76*, mentioned above, might also be examples of this phenomenon.

On closer examination, it will be noted that in most cases the cultures which were incubated in the dark have a little more growth than those incubated in the presence of light, suggesting that the intensity of the light source contributes to the inhibition of growth. Subsequent studies at 50 candles per square foot have shown that this is indeed true. Moreover, a strong light source bleaches the medium, making color interpretations between the light- and dark-incubated cultures even more difficult due to the lighter background of the light-incubated cultures. The growth difference also complicates the color interpretations.

TABLE 3

COLOR DEVELOPMENT IN THE PRESENCE OF LIGHT AFTER INITIAL ONE-WEEK INCUBATION IN THE DARK

Culture Number	1st Week Dark	2nd Week Light	3rd Week Light	4th Week Light
TAM 33	3+	3+	4+	4+
	b	o	r-o	r-o
TAM 60	4+	4+	4+	4+
	y-o	r-o	r-o	r-o
TAM 70	3+	3+	3+	4+
	y	y-o	y-o	y-o
TAM 59	3+	3+	4+	4+
	y	r-o	r-o	r-o
TAM 81	2+	3+	3+	4+
	y-o	r-o	r-o	r-o
TAM 21	2+	3+	3+	3+
	b	y	y	y
TAM 36	2+	3+	3+	4+
	b-y	y	y	y
TAM 25	3+	3+	3+	3+
	y	y	y	y
TAM 76	3+	3+	4+	4+
	y	r-o	r-o	r-o
TAM 27	2+	2+	3+	3+
	b-y	b-y	y	y
TAM 6	3+	3+	3+	3+
	b-i	b-c	b-ct	b-c
ORL 1	3+	3+	3+	3+
	b-i	b-c	b-tb	b-y-t
TAM 10	2+	2+	3+	3+
	b-i	y	y-yo	by-yo
TAM 16	3+	3+	4+	4+
	b-i	b-i	b-i	i-c
TAM 8	3+	3+	4+	4+
	b-i	b-c	b-ci	b-c

1+—50–200 colonies
2+—innumerable discrete colonies
3+—less than confluent
4+—confluent

y—yellow
o—orange
b—buff
r—red
t—tan

l—lemon
i—ivory
c—cream
k—khaki

TABLE 4

COLOR DEVELOPMENT IN THE PRESENCE OF LIGHT AFTER INITIAL TWO-WEEK INCUBATION IN THE DARK

Culture Number	2nd Week Dark	3rd Week Light	4th Week Light
TAM 33	3+	3+	4+
	b-y	r-o	r-o
TAM 60	3+	4+	4+
	r-o	r-o	r-o
TAM 70	4+	4+	4+
	y	y	y
TAM 59	3+	4+	4+
	y-o	y-o	y-o
TAM 81	3+	3+	3+
	y	y-o	y-o
TAM 21	3+	3+	3+
	b-y	y	y
TAM 36	3+	3+	3+
	y	y	y-yo
TAM 25	4+	4+	4+
	y	y	y
TAM 76	3+	4+	4+
	y	r-o-y	r-yo
TAM 27	3+	3+	3+
	b-y	by-y	y
TAM 6	3+	3+	3+
	b-i	i-c	i-c
ORL 1	3+	4+	4+
	b-c	c-t	b-c
TAM 10	3+	3+	3+
	b-y	by-y	b-y
TAM 16	3+	3+	4+
	b-i	i-c	i-c
TAM 8	3+	3+	4+
	b-i or c	b-i	i-c

1+—50–200 colonies
2+—innumerable discrete colonies
3+—less than confluent
4+—confluent

y—yellow
o—orange
b-buff
r—red
t—tan

l—lemon
i—ivory
c—cream
k—khaki

TABLE 5

COLOR DEVELOPMENT IN THE PRESENCE OF LIGHT AFTER INITIAL THREE-WEEK INCUBATION IN THE DARK

Culture Number	3rd Week Dark	4th Week Light
TAM 33	3+	4+
	y	o-ro
TAM 60	4+	4+
	yo-ro	r-o
TAM 70	4+	4+
	yo	y-o
TAM 59	4+	4+
	y-yo	y-o
TAM 81	4+	4+
	y-o	y-o
TAM 21	3+	3+
	t-y	by-y
TAM 36	3+	3+
	y	y
TAM 25	4+	4+
	y	y
TAM 76	3+	4+
	y-yo	y-o
TAM 27	3+	3+
	by	b-y
TAM 6	3+	3+
	i-t-c	b-c
ORL 1	3+	4+
	bc-t	b
TAM 10	3+	3+
	b-t-y	by-b
TAM 16	4+	4+
	b-i	i
TAM 8	4+	4+
	b-c	b-c

1+—50–200 colonies
2+—innumerable discrete colonies
3+—less than confluent
4+—confluent

y—yellow
o—orange
b—buff
r—red
t—tan

l—lemon
i—ivory
c—cream
k—khaki

In view of the limited data, certain conclusions are suggested regarding the growth and pigmentation of cultures incubated initially in the dark and subsequently in the presence of light. The results of these experiments are presented in tables 3, 4, and 5. In general, when a culture which had been incubated one, two, and three weeks in the dark was examined after the first week of light exposure, it was observed that it had taken on the pigment present in its light-incubated counterpart on the second week of light incubation. Furthermore, the pigment was quite stable during the remainder of the study period. Here again, the dark-incubated cultures demonstrate more growth than their respective light-incubated counterparts.

* * *

These foregoing observations suggest that the conditions under which the mycobacteria are incubated are significant in pigment production. Therefore, when using a grouping criterion based upon the degree of pigment production in the presence and absence of light, the conditions of exposure to light should be carefully controlled as to time and as to intensity of the light source. Unless standardized, these variables will lead to errors in interpretation. It is the practice in this laboratory to inoculate duplicate slants of Löwenstein-Jensen medium, one of which is incubated in the presence of light and the other in the dark. These are then examined between the second and third weeks.

Runyon's classification is sufficient in grouping the photochromogens and scotochromogens. It is easily understood why he classified the remaining unclassified mycobacteria generally as nonphotochromogens. Further subgrouping of the latter, based on pigment production, is not entirely adequate because of the borderline cases. It is convenient, however, for a tentative separation. Further studies along this line are under way.

ELDERT C. HARTWIG, JR.
Bureau of Laboratories, Florida State Board of Health, Jacksonville, Florida; and
Central Tuberculosis Laboratory, Southwest Florida Tuberculosis Hospital, Tampa, Florida

January 21, 1960

THE PRODUCTION OF "ANTI-ISONIAZID" SUBSTANCE BY ISONIAZID-SUSCEPTIBLE, ISONIAZID-RESISTANT, AND UNCLASSIFIED STRAINS OF MYCOBACTERIA[1]

Youmans and Youmans[2] reported that filtrates and extracts of laboratory strains of mycobacteria contained large amounts of a substance, apparently an enzyme, which would inactivate isoniazid *in vitro*. Later, it was found that this "anti-isoniazid" substance would also reverse the chemotherapeutic effect of isoniazid in tuberculous mice.[3]

In the course of these investigations, it was noted that the H37Rv strain which had been made resistant to isoniazid *in vitro* produced much less anti-isoniazid than several isoniazid-susceptible strains.[2] This isolated finding raised the question whether an inverse relationship might exist between resistance of mycobacteria to isoniazid and production of anti-isoniazid.

This paper is a report on the production of anti-isoniazid by recently isolated isoniazid-resistant and isoniazid-susceptible strains. Production of anti-isoniazid by several unclassified strains of mycobacteria and by the avirulent H37RaN strain also has been studied.

Ten isoniazid-resistant strains,[4] 6 unclassified strains,[5] 15 recently isolated human drug-susceptible strains,[6] the H37RaN strain,[7] and the isoniazid-susceptible H37Rv human strain of mycobacteria were employed. The strains were grown as surface pellicles on modified Proskauer and Beck medium[8] for five and eight weeks. At the end of each of these periods, filtrates of the cultures were obtained using a fine sintered glass filter.

The method used for measurement of the amount of "anti-isoniazid" in each filtrate has been described.[2] Briefly, it consists of adding 1.0 ml. of the sterile filtrate to sterile twofold dilutions of isoniazid made with the Proskauer and Beck medium. These tubes are inoculated with 0.1 ml. of 10^{-2} mg. of a finely ground suspension of the fast-growing saprophytic mycobacterial strain, 607. It had been found that the 607 strain and the H37Rv strain had similar endpoints when used as test organisms for the presence of anti-isoniazid[2] and, since the inhibitory activity of isoniazid tended to decrease during prolonged incubation, the faster growing 607 strain was selected. The tubes were incubated for four days, and the highest concentration of isoniazid in which growth occurred was recorded as the amount of isoniazid which was completely inactivated by the filtrate. A filtrate obtained from cultures of the H37Rv strain of the same age was included in each experiment as a control, and one to five tests were done with each culture filtrate. In addition, several five- or eight-week-old culture filtrates were obtained from most of the strains and were tested separately.

The mycobacterial strains, their susceptibilities to isoniazid, and the highest concentration of isoniazid which was neutralized by the five-week-old culture filtrates are listed in table 1. Large variation was encountered between the results of replicate tests done on filtrates obtained from eight-week-old cultures as compared with the uniform results obtained using filtrates from five-week-old cultures. This may be a reflection of the loss of "anti-isoniazid" which will occur in filtrates from older cultures.[2]

Also given in table 1 is the statistical significance of the difference between the sample means. There was no significant difference in the amount of anti-isoniazid produced by the isoniazid-susceptible and the unclassified strains. However, there was a significantly lower amount of anti-isoniazid produced by the isoniazid-resistant strains than by the drug-susceptible and the unclassified strains. Moreover, there was no significant correlation between production of anti-isoniazid and catalase activity.

A comparison was made between the amount of anti-isoniazid produced by the 4 strains which became resistant to isoniazid in the test tube or in the rabbit, and that produced by the 6 strains which became resistant in human beings. The lat-

[1] Aided, in part, by a research grant from the Tuberculosis Institute of Chicago and Cook County, Chicago, Illinois.

[2] YOUMANS, A. S., AND YOUMANS, G. P.: Am. Rev. Tuberc., 1955, *72*, 196.

[3] YOUMANS, A. S., AND YOUMANS, G. P.: Am. Rev. Tuberc., 1956, *73*, 764.

[4] Seven strains were obtained from Dr. William Steenken, Jr., Saranac Lake, New York; 2 from Dr. William Lester, Suburban Cook County Tuberculosis Association, Hinsdale, Illinois; and one from Dr. Milton Silverman, Veterans Administration Research Hospital, Chicago, Illinois.

[5] Obtained from Dr. Ernest Runyon, Veterans Administration Research Hospital, Salt Lake City, Utah.

[6] Obtained from Dr. Milton Silverman, Veterans Administration Research Hospital, Chicago, Illinois.

[7] YOUMANS, A. S., AND YOUMANS, G. P.: Proc. Exper. Biol. & Med., 1956, *92*, 566.

[8] YOUMANS, G. P., AND KARLSON, A. G.: Am. Rev. Tuberc., 1947, *55*, 529.

TABLE 1

THE EFFECT OF CULTURE FILTRATES OF STRAINS OF MYCOBACTERIA ON THE INHIBITORY ACTIVITY OF ISONIAZID

Human and Bovine Strains							Unclassified Strains		
Susceptible to Isoniazid			Resistant to Isoniazid						
Strain	Susceptibility of Strain to Isoniazid (Human Strains)	γ per Ml. of Isoniazid Inactivated by Culture Filtrate*	Strain	Catalase Activity	Susceptibility of Strain to Isoniazid	γ per Ml. of Isoniazid Inactivated by Culture Filtrate*	Strain	Susceptibility of Strain to Isoniazid	γ per Ml. o Isoniazid Inactivated by Culture Filtrate*
	γ per ml.				*γ per ml.*			*γ per ml.*	
124	0.045	31.0	1837 H†	+	1.9	7.8	1	0.045	11.7
135	0.022	54.0					2	0.045	4.4
157	0.022	39.0	#9 V.A.	+	3.9	5.8	5	1.9	13.6
166	0.022	31.0	series H†				26	1.9	36.6
231	0.045	19.0					146	0.1	28.0
235	0.045	17.0	9278-370 B‡	+	7.8	3.9	225	0.09	31.0
268	0.045	23.0							
322	0.045	31.0							
492	0.045	23.0	H1254 H†	+	7.8	15.6			
576	0.022	62.0							
595	0.022	31.0	9278-336 B‡	−	3.9	3.5			
705	0.045	31.0							
708	0.022	17.0							
718	0.045	17.0	Ravenel B§	−		3.9			
737	0.045	19.0							
H37Rv	0.045	86.0							
			H37Rv H§	−	3.9	7.8			
			6313 H†		1.9	15.6			
			6438 H†		7.8	7.8			
			62 H†		12.5	15.6			
		33.2				8.7			20.9
			P = <0.001				P = <0.005		
			P = <0.20, >0.10						

* Mycobacterium strain 607 was used as the indicator organism. The mean (18 experiments) bacteriostatic activity of isoniazid on this strain was 0.72γ per ml.

† Became resistant *in vivo* (humans).

‡ Became resistant *in vivo* (rabbits).

§ Became resistant *in vitro*.

\+ Some catalase activity.

− No catalase activity.

ter strains produced significantly more anti-isoniazid than the former strains (P = <0.025). However, these 6 strains still produced significantly less anti-isoniazid than the drug-susceptible strains isolated from humans (P = <0.02 > 0.01).

Although it had been noted previously that growth of the saprophytic 607 strain, the H37Rv strain, or the H37Rv strain-resistant to isoniazid in sub-bacteriostatic concentrations of isoniazid did not increase the yield of anti-isoniazid in the culture filtrates,[2] further attempts were made to increase the yield in this manner by using other myobacterial strains. Two isoniazid-resistant strains and 4 unclassified strains were grown on Proskauer and Beck medium in which isoniazid in a concentration of 0.01 γ per ml. had been incorporated. Filtrates from these cultures contained no more anti-isoniazid than did filtrates obtained from cultures grown at the same time on Proskauer and Beck medium that contained no isoniazid.

The H37RaN strain is a variant of the H37Rv strain which had become avirulent for guinea pigs and mice following serial transfer over a six-year

period on a nitrogen-free Proskauer and Beck medium.[7] This strain was transferred once to a complete Proskauer and Beck medium in order to obtain a heavy pellicle growth, and filtrates of these cultures were tested for anti-isoniazid. The results of several such determinations revealed that the H37RaN strain produced from one-tenth to one-fiftieth the amount of anti-isoniazid as did the parent H37Rv culture, even though the H37RaN culture was just as susceptible to the growth-inhibitory action of isoniazid as was the H37Rv culture.

* * *

Isoniazid-susceptible mycobacterial strains, by virtue of some unknown metabolic process, are able to produce a substance, "anti-isoniazid," which will inactivate the growth-inhibitory action of isoniazid. On the other hand, strains of mycobacteria which have become resistant to isoniazid, and are therefore metabolically changed, produce very much less anti-isoniazid than do susceptible strains. Furthermore, this change is more pronounced in strains which become resistant to isoniazid *in vitro* or in rabbits than in strains which become resistant following exposure to isoniazid in humans.

Since anti-isoniazid is formed in the absence of isoniazid and addition of isoniazid to the culture medium does not increase its production, it apparently is not an adaptive enzyme.

Production of a fairly large amount of anti-isoniazid by the H37Rv strain may provide an explanation for some of the findings of Barclay and co-workers[9] who, using C^{14}-labeled isoniazid, reported that more isoniazid was bound when cells of this strain were maintained at 5°C. At this temperature the metabolic activities of the cells would be greatly reduced and less anti-isoniazid would be formed than at the optimal growth temperature, 37°C. Moreover, these investigators found that, after an early rapid fixation of isoniazid which reached a maximum in sixteen to twenty-four hours, there was a 20 to 50 per cent decrease of the bound isoniazid. They were unable to decide whether this decline was due to the increase in cell mass or "actual loss of isoniazid that had been bound." It is possible that the bound isoniazid was actually lost due to the anti-isoniazid combining with the isoniazid to form a compound which would not be bound by the cell. In this connection, Barclay and his associates also tested three C^{14}-labeled compounds which resembled isoniazid in their chemical structure, and found very little of these compounds bound by the cells.

The unclassified mycobacteria produced as much anti-isoniazid as did the drug-susceptible human strains, although 2 of the unclassified mycobacteria were resistant to isoniazid. These resistant strains came from human patients, and resistant strains isolated from humans have been shown to produce more anti-isoniazid than do resistant strains produced *in vitro*.

Both anti-isoniazid, which inactivates isoniazid and apparently is an enzyme,[2] and penicillinase, the enzyme which inactivates penicillin, are produced in greater amounts by strains which become resistant in humans than those which become resistant *in vitro*.[10-12] In contrast to the findings with anti-isoniazid, however, penicillin-susceptible strains of staphylococci do not appear to produce penicillinase, while resistant strains do produce it.[13-15] Moreover, penicillinase has been shown to be an adaptive enzyme[16] which anti-isoniazid apparently is not.

In summary: Culture filtrates of 10 isoniazid-resistant strains, 6 unclassified strains, 15 recently isolated human drug-susceptible strains, the H37RaN strain, and the H37Rv strain were tested for the presence of "anti-isoniazid." It was found that the unclassified and drug-susceptible strains produced a significantly larger amount of anti-isoniazid than did the resistant strains and the H37RaN strain. There was no relationship between the production of anti-isoniazid and the catalase activity of the resistant strains, but more anti-isoniazid was produced by resistant strains isolated from humans than from strains which developed resistance *in vitro* or in the rabbit. The presence of isoniazid in the culture medium did not increase production of anti-isoniazid.

ANNE S. YOUMANS
GUY P. YOUMANS
Department of Microbiology
Northwestern University Medical School
Chicago, Illinois

January 27, 1960

[9] BARCLAY, W. R., *et al.*: Am. Rev. Tuberc., 1954, *70*, 784.

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Correspondence

COMMUNITY TRIALS OF BCG VACCINATION

To the Editor of The American Review of Respiratory Diseases:

In your journal of June, 1958, a paper on community trials of BCG vaccination is presented (by C. E. Palmer, L. W. Shaw, and G. W. Comstock).

Here, far less effect of the vaccination is found than that which has been demonstrated in other parts of the world. Before the reasons for this discrepancy may be further evaluated, it would be valuable for the readers to have information on the following points:

(1) How reliable were the vital statistics in Puerto Rico in the period in question, particularly regarding tuberculosis morbidity?

(2) What were the qualifications of the personnel and how was the technical equipment available in the 20 different tuberculosis centers in Puerto Rico, giving the basis for the figures in the paper?

(3) What were the detailed criteria for the diagnosis of primary tuberculosis in the vaccinated group in Puerto Rico (particularly with reference to the statement in the paper that the diagnosis of pulmonary tuberculosis is difficult in vaccinated individuals)?

(4) How would the removal of the 24 cases of primary tuberculosis from the 94 cases of tuberculous disease in the vaccinated group affect the total figures and the conclusions of the paper? Can a case be made for the inclusion of these 24 cases?

(5) How many of the 23 cases of minimal tuberculosis among the vaccinated had the diagnosis verified by bacteriologic findings, et cetera, and not only indicated by the tuberculin reaction? What have the authors done to analyze critically the cases of minimal tuberculosis in order to make the comparison between the groups valid?

(6) Which precautions were taken in Puerto Rico to protect the vaccine from the effect of sunlight? If no precautions were taken, do the authors think that the use of sun-damaged vaccine may account for some of the poor results of the vaccination?

(7) With the serious defect of the trial—no information about the tuberculin reaction shortly after the vaccination—how can the possibility be excluded that a large proportion of the vaccinated individuals had not obtained a positive reaction to tuberculin because of the vaccination, but only because of infection with virulent, human tubercle bacilli in the period (as is strongly indicated by comparison with the Muscogee-Russell figures)?

(8) Thus, how many of the observed cases of tuberculosis in the "vaccinated" group occurred in "improperly" vaccinated and how many in "properly" vaccinated individuals?

Answers to questions like the ones above will—to readers outside the United States anyhow—be important in allaying doubts as to the significance of this paper on community trials of BCG vaccination.

EILERT EILERTSEN
Bergen Helserâd, Bergen, Norway.

January 8, 1960.

To the Editor of The American Review of Respiratory Diseases:

Throughout Dr. Eilertsen's letter one senses a much greater concern for the precise evaluation of cases among the vaccinated group than for the validity of the comparisons between various components of the study population. Our emphasis was exactly the opposite—without belittling precision, we thought that handling the entire study population in a similar fashion should be paramount.

Questions (1) and (2) by Dr. Eilertsen may be answered together. Obviously, we believed that tuberculosis case reporting and diagnosis in Puerto Rico were adequate for our purpose. It is equally obvious that we did not consider either function perfectly precise and complete, but it is unlikely that perfection in these matters is achieved by any of us in any country. As we pointed out several times, the important issue seemed to be that of bias. Great pains were taken to ensure that the status of the individuals in the trials—reactors, controls, and vaccinees—could not influence the subsequent diagnosis of tuberculosis. Both the British and our trials indicated that BCG was about equally effective in preventing both severe and slight disease. Consequently, a few moments spent in reflection will show that the same *relative* risks for the three groups, and the same percentage reduction in tuberculosis attributable to vaccination, will be found if any fraction of the total cases is reported, as long as that fraction is unbiased, i.e., the same for all three groups.

Regarding questions (3), (4) and (5), the answers can be obtained simply by working out the rates from the published tables, omitting primary

and minimal cases. For instance, if only advanced pulmonary tuberculosis, miliary and meningeal tuberculosis, and bone and joint tuberculosis are cousidered suitable for analysis, the percentage reduction in tuberculosis among the vaccinated as compared with the controls is essentially unchanged, namely, 35 per cent for Puerto Rico and 33 per cent for Muscogee and Russell counties. This point was discussed in our publication.

As for question (6), the vaccine was protected from sunlight throughout the Puerto Rico trial even though the deleterious effect of sunlight on BCG vaccine was not known until the summer of 1951. Protection against sunlight was achieved indirectly as a result of attempts to protect the vaccine against heat. Tuberculin testing of vaccinees six to thirteen months after vaccination showed that 93 per cent of them were then reactors to the 10 TU dose.

Question (7) implies that the vaccinees in Puerto Rico experienced a high conversion rate attributable to infection with *M. tuberculosis*. In our paper we gave evidence that the infection rate following the trial did not appear to be high in Puerto Rico, although it was undoubtedly higher than in Muscogee and Russell counties. There is no reason to believe that the control group is dissimilar from the vaccinated group except for the fact of vaccination. Consequently, it seems reasonable to attribute most of the high conversion rate among vaccinees to vaccination, since their rate was so much higher than that among controls tested at the same time.

The answer to question (8) can also be obtained from an analysis of the published data. Approximately 90 per cent of the vaccinees were reported to have converted to the 10 TU dose of PPD one to two years after vaccination (it was slightly higher at an earlier period). If one wishes to adopt the extreme view that the 10 per cent who did not react at that time were not only "improperly" vaccinated, but essentially unvaccinated, one would expect the control rate of developing tuberculosis to apply to them. Then the rate among the "properly" vaccinated would have been 25.5 per 100,000 per year instead of 29.8 as reported for the entire group of vaccinees. This is equivalent to a reduction in tuberculosis among the "properly" vaccinated of 41 per cent.

Surely it should be clear that science is not served by a critical analysis of the cases from only one part of the study population, the vaccinees. There can be no objection if someone wishes to disregard a particular category of cases as long as *all* the cases of that category are disregarded, and not merely those from one part of the population. It was the latter kind of "critical analysis"—such as "the removal of the 24 cases of primary tuberculosis from the 94 (sic) cases of tuberculous disease in the vaccinated group" with no mention of removing them from the other study groups—that kept BCG vaccination in disrepute during its early years.

GEORGE W. COMSTOCK
Washington, D. C.

March 2, 1960

Books

RECENT TRENDS IN CHRONIC BRONCHITIS. Edited by Neville C. Oswald. Pp. 199, Oxford University Press, New York, 1958. Price, $7.50.

Chronic bronchitis as a disease entity exists to a greater or lesser extent in many parts of the world, although its true prevalence is unknown. This ignorance is due to many factors which stem mainly from the very protracted course of the disease and from the difficulty in distinguishing its later stages from other diseases causing obstruction of the airways and emphysema. The pressing problem is one of definition and, until this is settled, the natural history of the disease will remain a mystery, and misunderstandings existing between various centers actively interested in the problem of chronic bronchitis will persist.

Gaensler has recently shown, after reassessing more than 500 patients with diffuse obstructive emphysema in New England, that about two-thirds had productive cough for a considerable period before the onset of breathlessness. This large group of cases, therefore, fell within the definition of chronic bronchitis generally accepted in the United Kingdom where Fletcher, adopting an epidemiologic approach, has defined bronchitis as "that condition existing when a cough is accompanied by the persistent expectoration of sputum at least once daily on most days at least three months in each year, when no other disease is present; complications giving rise to disability in the form of recurrent infection and breathlessness are frequent." In an early chapter in the book under review, breathlessness is included in the definition of the disease and, therefore, it is clear that the subject matter is primarily concerned with the condition after it has become well established. Epidemiology and causation have not been the chief concern of the writers.

This book brings together the experience of several workers interested in different aspects of the condition under the editorship of Dr. Neville Oswald from the Brompton Hospital in London. Here, as in the rest of the United Kingdom, the incidence of chronic bronchitis is very high and causes a considerable morbidity and fatality. In this book the reader will find considerable information about the established disease and its complications, with notable contributions on the pathology, roentgenography, bacteriology, physiology, and management (including that in general practice). Each chapter contains many pertinent references.

This work should prove very valuable to both general physicians and chest specialists who do not have a clear appreciation of the disease. The knowledge gained should prove a stimulus to research into the cause and prevention of a disease in which treatment has so little effect.

JOHN BATTEN
St. George's Hospital
London, England

ADVANCES IN TUBERCULOSIS RESEARCH, Volume IX. Edited by Hans Birkhauser, Hubert Bloch, and G. Canetti. Pp. 343, S. Karger, Basel, Switzerland (available through Albert J. Phiebig, White Plains, New York), 1958. Price, $19.20.

This volume maintains the high standard of its predecessors. There are seven papers on widely different aspects of tuberculosis contributed by experts from America, Canada, France, Germany, Japan, Russia, and Sweden.

Tuberculosis in Our Time (in English). E. R. Long, U. S. A.: A review is given of the factors which have resulted in the decline of tuberculosis in the United States over the last seventy years. Forecasting the future, based on the latest death rates in cohorts, it is estimated that 12,000 deaths from tuberculosis may occur in 1977 in the United States, with 44,000 new active cases and 33 million tuberculin reactors. These are no mean figures and indicate the need for continuing an efficient organization for tuberculosis control, which at present costs 725 million dollars a year.

The Pathogenesis of Tuberculosis Cavities (in English). Y. Yamamura, Japan: An experimental method is described for the production and study of tuberculous cavities in rabbits. Tuberculous allergy plays an important role in cavity formation; the sensitizing component is a phospholipid derived from tubercle bacilli. Desensitization prevents cavity formation.

Patho-Histological Changes in the Nervous System in Various Forms of Tuberculosis in Man (in English). V. I. Puski and D. A. Uvarova, U. S. S. R.: A systematic study was made of the central nervous system in 70 patients dying from tuberculosis. Most of the lesions occurred in hematogenous forms of tuberculosis, resulting in pronounced proliferative changes in the obliterated blood vessels and glia. These changes were most marked in the region of the hypothalamic nuclei and in the oblongata. In the cerebro-

spinal cord, the nerve cells of the lateral columns were mainly affected.

Lymphogranulomatosis Benigna (Morbus Boeck-Schaumann) and Atypical Tuberculosis (Ziegler), A Study of their Relationship (in English). L. Zettergren: This study was undertaken to determine the relationship between sarcoidosis (lymphogranulomatosis benigna) and tuberculosis, including Ziegler's atypical chronic hyperplastic form of tuberculosis. This stimulating investigation was based on the study of a large number of lymph nodes showing tuberculoid lesions. The lymph nodes were divided into two series according to whether or not acid-fast bacilli were present in the sections. There were 59 nodes in which bacilli were found and which were representative of the Ziegler form of tuberculosis. In the other series, without bacilli (53 nodes), the clinical picture was consistent with lymphogranulomatosis benigna and differed clinically from the Ziegler form of disease. Histopathologically, however, the two series were alike. The author concludes, therefore, that lymphogranulomatosis benigna is a tuberculous disease and that the Ziegler form of tuberculosis is a transitional form between it and classical tuberculosis.

Experimental Murine Tuberculosis for Screening Antituberculous Compounds (in German). W. H. Wagner, Germany: A very detailed review is given of the world literature on experimental tuberculosis in the mouse, covering both the pathology of the disease and the use of murine tuberculosis for assessing antituberculous agents. The various methods used are critically analyzed. The author, quite rightly, points out that murine tuberculosis is essentially different from tuberculosis in the guinea pig and in man.

Enzyme Deficiencies in Isoniazid-resistant Tubercle Bacilli (in French). B. Kreis, France: The author combines a lucid review of the extensive and controversial literature, with a report of original investigations on the subject. Isoniazid-resistant tubercle bacilli are deficient in two of the iron porphyrin respiratory enzymes, namely, catalase and peroxidase and, frequently, also urease. The author suggests that isoniazid exerts its action by breaking an enzymatic chain reaction, perhaps at the stage of peroxidase, since isoniazid-resistant bacilli are generally peroxidase deficient. The deficiencies in catalase and peroxidase are not proportionate, however, to the degree of resistance or to the virulence of the organisms.

The Use of Radioactive Isotopes in the Study of Experimental Tuberculosis (in English). J. Sternberg, Canada: This paper is of the greatest value because it presents concisely and critically the application of radioactive isotopes to the study of experimental tuberculosis; a complete list of references to both the basic principles and specific applications of radioisotope techniques are provided. *In vitro* and *in vivo* studies with labeled myobacteria have been used to study both the metabolism of the bacilli and the effect of drugs on their metabolism. *In vivo* studies with labeled bacilli have provided quantitative information on the dispersion of the organisms in the host tissues and biochemical exchanges between the bacilli and the infected host. The mode of action of antituberculous drugs has also been studied by using labeled bacilli or labeled drugs, both *in vitro* and *in vivo*. Furthermore, the use of labeled drugs can be used to study more precisely the distribution of the drugs in both normal and infected animals.

R. J. W. Rees
National Institute for Medical Research
London, England

BOOKS RECEIVED

Current Therapy—1960: Latest Approved Methods of Treatment for the Practicing Physician. Edited by Howard F. Conn. Pp. 808, W. B. Saunders Company, Philadelphia and London, 1960. Price, $12.00.

Antibiotica et Chemotherapia. Vol. 7. Fortschritte—Advances—Progrès. Edited by O. Gsell. Pp. 374, S. Karger AG, Basel and New York, 1960. Price, Sfr. 64.

Encyclopedia of Urology: Inflammation II. Specific Inflammations. By Einar Ljunggren, R. Campbell Begg, and Ambrose J. King. Pp. 564, Springer-Verlag, Berlin, Göttingen, Heidelberg, 1959. Price, DM 158.

Year Book of Drug Therapy (1959–60 Series). Edited by Harry Beckman. Pp. 570, Year Book Publishers, Inc., Chicago, 1960. Price, $8.50.

Recent Advances in Public Health. By J. L. Burn. Pp. 370, Little, Brown and Company, Boston, 1960. Price, $10.00.

Traitement de L'Insuffisance Respiratoire. Rapports Présentés au XXXIIe Congrès Français de Médecine, Lausanne, 1959. Pp. 334, Masson et Cie., Paris, 1959. Price, 35 NF.

Laboratory Manual and Workbook in Microbiology. For Students of Nursing. By Lucille Sommermeyer. Pp. 154, W. B. Saunders Company, Philadelphia and London, 1960. Price $3.50.

American Trudeau Society

Medical Section of the National Tuberculosis Association

ABSTRACTS OF PAPERS PRESENTED AT THE ANNUAL MEETING OF THE AMERICAN TRUDEAU SOCIETY HELD IN CONJUNCTION WITH THE MEETINGS OF THE NATIONAL TUBERCULOSIS ASSOCIATION AND THE NATIONAL CONFERENCE OF TUBERCULOSIS WORKERS, MAY 15–20, 1960

1A The Differentiation of Mycobacteria

Changes in the Tuberculin Pattern in Medical Students between 1930 and 1960

David T. Smith and William W. Johnston, Durham, North Carolina

The precipitous drop between 1930 and 1960 in the percentage of medical students reacting positively to tuberculin is a reflection of equally dramatic changes in the socio-economic and tuberculosis control patterns between 1920 and 1950.

Between 1930 and 1940 approximately 60 per cent of the second-year medical students at Duke University gave positive tuberculin reactions to 1:1,000 OT, and 20 per cent reacted to only 1:100 OT. During the following years there was a progressive reduction in the number reacting to only 1:1,000 OT and a surprisingly steady increase in the number reacting to 1:100 OT. In 1956–1957, among 144 medical students, 35 (24 per cent) reacted positively to 1:1,000 OT, 54 (38 per cent) were positive to 1:100 OT, and 55 (38 per cent) were negative. Among 141 medical students tested in 1958–1959, 11 (8 per cent) were positive to 5 TU PPD, 55 (39 per cent) reacted to 1:100 OT, and 75 (53 per cent) were negative.

In the 1958–1959 classes, 151 students were tested with 5 TU of a PPD made from the Battey atypical organism and 5 TU made from an avian tubercle bacillus. Of those tested, 38 (25 per cent) gave reactions of 6 mm. or more to the Battey tuberculin, and 30 (20 per cent) gave similar reactions to avian PPD. In 1959, 14 students failed to react to 1:100 OT or to 0.005 mg. of avian PPD. All were retested with 1:10 OT and found to be negative. In contrast, 22 students were negative to 1:100 OT but positive to 0.005 mg. of avian PPD. All were retested with 1:10 OT, and 9 were found to have from 6- to 30-mm. reactions, 4 had 3- to 4-mm. reactions, and 10 gave zero reactions. Of the 55 students who took the entire series of tests, 6 were positive to 5-TU PPD, 13 positive to 1:100 OT, and 14 (25 per cent) reacted positively to 0.005 mg. of avian PPD but failed to react to 1:10 OT. Some evidence suggests that these students may have been infected with the avian tubercle bacillus.

A Study of Reactions to Various Anonymous Mycobacterial Antigens in Dallas County Orphans

Hal J. Dewlett, L. Lefkowitz, R. Fulayfil, Lynn C. Christianson, and John S. Chapman, Dallas, Texas

Five hundred and six children, ranging in age from one to twenty years, were given Mantoux tests with Old Tuberculin and with tuberculins from the three anonymous mycobacteria in a 1:1,000 dilution.

Forty-four children had reactions of 10 mm. or more to one or more antigens. Forty-one (8.1 per cent) reacted to the photochromogen, 9 (1.8 per cent) to the scotochromogen, 8 (1.6 per cent) to OT, and 2 (0.4 per cent) to the nonchromogen.

Those positive to the anonymous mycobacterial antigens were concentrated in the 7 to 14 age group.

Results of the chest roentgenograms are available for 40 of the 44 reactors. In none was there an active lesion, although abnormalities were present in 27. Twenty-five (62.5 per cent) had calcified primary lesions, and 2 (5 per cent) demonstrated other parenchymal residua.

Twenty-three (92 per cent) of the 25 with calcified primary lesions reacted to the antigens derived from anonymous mycobacteria. Six (24 per cent) reacted to OT, and 11 (44 per cent) reacted to histoplasmin.

The reactions to the photochromogenic antigen were fivefold greater than to a comparable concentration of OT. The paucity of reactors to the nonchromogenic antigen parallels the results obtained with these same antigens in adults. The infrequency of cross reactions between OT and photochromogen antigen in these children is in contrast to the results of our studies in adults. Of particular significance was the finding of 11 children (44 per cent) with calcified primary lesions who reacted only to the antigens from anonymous mycobacteria.

Epidemiologic Interpretation of Comparative Tuberculin Testing

H. Mac Vandiviere, M. R. Vandiviere, and H. S. Willis, Chapel Hill, North Carolina

This is an epidemiologic study and interpretation of tuberculin sensitivity patterns in several

separate and distinct populations. These include (*1*) tuberculous patients, (*2*) tuberculin-negative individuals who were subsequently vaccinated with the attenuated human variant R_1, (*3*) tuberculin-negative individuals subsequently vaccinated with the attenuated bovine variant BCG, and (*4*) healthy individuals with naturally acquired tuberculin sensitivity. Approximately 2,000 people were tested, and about 6,000 tests were made.

Antigens tested were the international tuberculin, PPD-S, purified tuberculoproteins (type A, type B, and type C) isolated from unheated culture filtrate from a human strain of tubercle bacillus, and a bacillary protein. In each case all of the antigen used was from a single lot prepared by Dr. Florence Seibert, lyophilized in quantities needed for a survey, and requiring only the addition of buffer solution immediately before use. Concentrations were 0.005 mg., 0.0001 mg., and 0.00002 mg. per dose, the tuberculoprotein being administered on one arm and the corresponding dose of PPD-S on the opposite arm as a control in each case. In addition, tests were made comparing avian tuberculin, Battey PPD, and PPD-S. All reactions were measured after 48 hours as two diameters, and the thicknesses and volumes were calculated as the segment of a sphere. Uniform techniques and procedures were enforced throughout the study.

Cross-reactions or nonspecific reactions are known to occur frequently among populations tested with tuberculins in general use, especially among those subjects exhibiting low-grade sensitivity. The best tuberculin in use, even though markedly free from impurities, has been found to be a mixture of proteins. These have been isolated and tested to determine if a single tuberculoprotein exists that can detect true reactors with greater specificity and/or sensitivity than the mixed antigens now in use.

Histograms have been prepared that show frequency distributions of sizes of reactions to each type of antigen at each concentration tested in tuberculous patients, in R_1-vaccinated, in BCG-vaccinated, and in naturally sensitized healthy subjects.

Correlations of sizes of reactions to each concentration of PPD-S tested with each of the other antigens tested for the various populations have been prepared.

The data have been carefully analyzed statistically. Interesting epidemiologic interpretations have resulted.

Outstanding is the dominance of PPD-S reactions in those of low hypersensitivity compared with the dominance of protein type B reactions in those of high sensitivity.

Antigenic Response to Mycobacterial Infection

CARROLL E. PALMER, LYDIA B. EDWARDS, Washington, D. C., LEWIS F. AFFRONTI, Chamblee, Georgia, LOUISE HOPWOOD and PHYLLIS Q. EDWARDS, Washington, D. C.

Epidemiologic studies of natural sources of tuberculin cross reactions and techniques for identifying cross reactions are supplemented by studies of guinea pigs inoculated with atypical mycobacteria isolated from human patients and tested simultaneously with PPD tuberculin from human tubercle bacilli and PPD antigens prepared from the inoculated strains. Doses of the antigens were adjusted to contain equivalent amounts of protein nitrogen.

Most strains produced transient local lesions, but none caused progressive systemic disease. Sensitivity to the homologous antigen (prepared from the inoculated strain) developed in nearly every animal; the average size as well as the distributions of reactions for each group were strikingly alike. Reactions to heterologous antigens varied widely in frequency.

Because the reaction was generally larger to the homologous than to the heterologous antigens, the source of sensitivity could be correctly identified in a high proportion of individual animals, provided the homologous antigen for each group was included as one of the test antigens. Accuracy of identification decreased, however, as animals sensitized by organisms not represented among the test antigens were added to the study population.

Intracellular Growth of Tubercle Bacilli and Other Mycobacteria in the J-111 Cell Line of Leukemic Monocytes

EDWIN A. BROSBE, PAUL I. SUGIHARA, and C. RICHARD SMITH, Long Beach, California

J-111 cells were cultured on coverslips in Leighton tubes, employing Eagle's basal medium supplemented with serum from various sources. The vigorously growing cells showed ameboid activity and readily phagocytized mycobacteria when the medium contained horse or lamb serum.

Twelve strains of *Mycobacterium tuberculosis*, isolated from 6 patients before and during chemotherapy, grew within the cytoplasm at approximately the same rate. Five of the chemotherapy strains had impaired virulence for guinea pigs. All strains appeared to form strong cores.

Two BCG strains and the H37Ra strain of *M. tuberculosis* grew at a much slower rate and did not form true cords.

Two strains of Group I photochromogenic mycobacteria grew intracellularly at a rate closer to that of *M. tuberculosis*. The bacilli were en-

larged, especially elongated, and displayed characteristic transverse barring. Although there was an inconstant tendency to parallel alignment, this may have been due to limitations of the intracellular space.

Two strains of Group III nonphotochromogenic mycobacteria grew at a faster rate and showed evidence of branching.

Our observations were similar to those reported by Shepard on the behavior of various mycobacteria in HeLa cells.

1B Emphysema

The Elastin and Collagen Content of Normal and Emphysematous Human Lungs

GEORGE W. WRIGHT, JEROME KLEINERMAN, and ELINOR M. ZORN, Cleveland, Ohio

Alteration of the elastic properties of the lung in diffuse obstructive emphysema has been observed, and alteration of the elastic tissue has been offered in explanation. The purpose of this study was to determine elastin and collagen content of normal human lungs of various ages and compare these data with those from lungs of persons with diffuse obstructive emphysema. Lungs were washed free of blood, freeze-dried, and lipid-extracted. Collagen was removed from the dry fat-free lung by repeated autoclaving until the aqueous extract was biuret negative. The remaining substance was the elastin residue. Hydroxyproline and hexosamine analyses were performed on hydrolysates of the elastin residue and dry fat-free lung. From these data, elastin and collagen contents were determined.

Twenty-two normal and seven diseased lungs were studied. The diseased lungs were from persons clinically, roentgenographically, and physiologically defined as having diffuse obstructive emphysema. No consistent variation in elastin content was noted between right and left lungs or between upper and lower lobar divisions of normal lungs. There was a significant increase in elastin content of lungs of older normal persons ($r = 0.588$). Collagen content and total fibrous protein showed no distinct alteration with age. Seven emphysematous lungs were no different in elastin and collagen content than normal lungs in persons of similar age. There was a decrease in the hexosamine content of the elastin fraction with advancing age, and the emphysematous specimens did not vary from the normal pattern. Our data indicate that elastin expressed as a percentage of dry fat-free lung weight increases with age. The elastin content of lungs physiologically defined as having diffuse obstructive emphysema was no different from the elastin content of normal lungs from persons of similar ages. This would suggest that diffuse obstructive emphysema is not caused by a specific elastin-destroying process.

Prognostic Factors in Pulmonary Emphysema

GEORGE BOWER, Denver, Colorado

One lesion of emphysema is destructive of capillary-containing alveolar walls, which contributes to impaired gas exchange and cor pulmonale.

Fifty emphysematous subjects were followed for as long as two and a half years. All had moderate or severe disease judged by ventilatory impairment and increase in residual volume/total lung capacity (RV/TLC). Arterial blood gas and diffusing capacity (D_{CO}) determinations were made during rest and exercise.

The 10 subjects who died during follow-up differed little from the survivors with respect to ventilatory function. Mean RV/TLC was somewhat higher, hypoxemia and hypercapnia were more pronounced, and mean percentage of CO extracted from that presented and exercise D_{CO} were significantly lower in those who died. Weight loss, polycythemia, and/or heart failure were common in the 10. The lungs of 7 who died were examined; all showed emphysema.

Half of the 50 subjects extracted less than 22 per cent of the CO presented. Nearly all deaths were in this group; hospitalizations and inability to work were far more common among them.

Impaired ability to extract CO did not parallel that of ventilation, suggesting that the former may be a better guide in estimating the severity of emphysema.

The Incidence of Obstructive Emphysema in Patients with Tuberculosis

W. Y. HALLETT and C. J. MARTIN, Seattle, Washington

All admissions to Firland Sanatorium over a one-year period were examined for the diffuse obstructive pulmonary syndrome (emphysema of the generalized obstructive type) by measuring maximal expiratory flow (MEF) rates. All were carefully questioned regarding symptoms, history of respiratory disease, occupational exposure to possible respiratory irritants, use of tobacco, coexistence of other diseases, et cetera. Patients found to have low expiratory flow rates were further tested by other ventilatory measurements. Arterial blood gases and pH were measured when airway obstruction was severe.

In more than 700 consecutive routine admissions (readmissions and children excluded) during this period one-third (34 per cent) were found to have diffuse obstructive pulmonary disease. Of

those with obstructive disease, 46 per cent were asymptomatic, 29 per cent were moderately obstructed, and 25 per cent were severely obstructed, often with hypoxia and hypercarbia.

There was a striking correlation of the diffuse obstructive pulmonary syndrome with age, the incidence of the former being negligible before 30 years, but present in more than 50 per cent of those older than 50 years.

A similar relationship existed between the severity of pulmonary tuberculosis and the degree and incidence of obstruction. Of the patients with far advanced pulmonary tuberculosis, 57 per cent had obstructive disease; the mean maximal expiratory flow rate of this group was 214 L/min. In those with minimal tuberculosis, the mean MEF was 295 L/min., and the incidence of obstructive emphysema was 22 per cent. This relationship appears to be exclusive of age and gives rise to speculation as to its nature. Is one condition a cause and the other an effect?

Smoking, which has been found by others to be so closely related to the diffuse obstructive pulmonary syndrome, bore no such relationship in this group of tuberculous patients. No correlation between duration of smoking or amount smoked and degree of obstruction could be demonstrated. Many other factors (occupation, sputum, cough severity, disease of certain organ systems, frequent "pneumonia," et cetera) were found to have a relationship to emphysema.

The Incidence of Diffuse Airway Obstruction in an Industrial Population

GEOFFREY L. BRINKMAN and E. OSBORNE COATES, JR., Detroit, Michigan, and DUANE L. BLOCK and H. J. VAN VALZAN, Dearborn, Michigan

The vital capacity, first- and third-second vital capacity, and maximal midexpiratory flow were determined in 1,341 men aged 40–65. These men were working full-time in one of the following occupational groups: no industrial exposure, industrial but no silica exposure, silica exposure but no X-ray evidence of silicosis, and silica exposure and X-ray evidence of silicosis.

Normal values for the above spirographic parameters are established, and comparison made between values obtained by two spirometers in common use. The adverse influence of increasing age, occupation, dust exposure, silicosis, smoking, and bronchitis on the spirogram has been demonstrated and the incidence of diffuse airway obstruction in the male population estimated.

Evidence shows that an ideal survey technique for determining diffuse airway obstruction has yet to be developed.

Comparison of Lung Function Tests Based on Maximal Expiratory Effort

JOHN R. GOLDSMITH, Berkeley, California

In a number of epidemiologic studies, the pulmonary function of various populations was assessed. A variety of physiologic tests were used, including vital capacity, forced expiratory volume and measurements derived from the curve, indicating maximal expiratory flow using a laminar flow resistor (pneumometer), and indicating maximal expiratory flow using a vane anemometer (Wright peak flow meter).

Comparison of the various tests for epidemiologic and screening studies was the major consideration. The following are the criteria used: simplicity of performance for the technician and subject, variability with repeated determinations, relationship between pulmonary function tests and concomitant physical characteristics (age, height, et cetera), influence of pulmonary conditions on pulmonary function tests, influence of smoking and air pollutants on pulmonary function tests, and suitability for population screening studies.

The indicating maximal expiratory flow tests are simplest to use. The laminar flow resistor, used in thousands of tests, proved to be rugged and easily cleaned. The vane anemometer is not as simple or as readily cleaned but it has the advantage of retaining its maximal reading.

The forced expiratory volume in one second and both indicating maximal expiratory flow tests appear suitable for screening purposes.

A Feasible and Accurate Method for Mass Pulmonary Function Screening

BRIGITTE B. NAHMIAS, ROSS L. MCLEAN, and A. GRIGG CHURCHWELL, Atlanta, Georgia

In 1959, a survey was made of the respiratory system abnormalities of 344 working adults in a rural Georgia community. This group, constituting the control population of an industrial study, included 308 men and 37 women of various occupations and ranging in age from 18 to 67 years (mean: 40). This survey included respiratory system history and physical examination, chest X-rays, skin tests, and specific spirometric studies. To our knowledge this is the first time a Stead-Wells spirometer was used in a community survey. Utilizing the reduced resistance, lessened dead space, and the fast drum speed of this instrument, we obtained permanent records of vital capacity and expiratory flow rate.

Of the 344 subjects, 200 were considered to have no respiratory abnormality by X-ray, history, or physical examination. These constitute our "nor-

mal" population. The remaining 144 subjects constitute our "abnormal" population. Vital capacity and maximal mid-third expiratory flow rate values have been prepared for both groups by age, sex, race, and occupation. Particular interest was placed upon the maximal mid-third expiratory flow rate (MM ⅓ EFR). The value and characteristics of this previously unreported measurement and normal and abnormal pulmonary function tests have been studied in relation to smoking, X-ray abnormalities, and pulmonary symptoms.

In summary, we present an accurate, feasible method for mass pulmonary function screening; normal values for an adult working population of vital capacity and the maximal mid-third expiratory flow rate; and salient correlations of vital capacity and MM ⅓ EFR to respiratory symptoms, habits, and X-ray findings.

2A Clinical Chemotherapy

A Double-blind Trial of Prednisolone in the Treatment of Pulmonary Tuberculosis

SHIRLEY H. FEREBEE, Washington, D. C.

This paper is based on the experience of more than 1,800 patients in 25 hospitals observed for 32 weeks in a double-blind trial of prednisolone in five- and nine-week courses and matching courses of placebo. Chemotherapy was randomly assigned, each patient receiving either 32 weeks of isoniazid plus PAS or 12 weeks of daily streptomycin plus pyrazinamide followed by 20 weeks of isoniazid plus PAS.

Preliminary observations after 12 weeks indicate that both five- and nine-week courses of steroid accelerated X-ray clearing. Patients receiving a five-week course of prednisolone became sputum negative at the same rate as those receiving placebo, but those receiving an eight-week course of prednisolone did less well bacteriologically.

By the time of the annual meeting, all patients will have completed their 32-week course of treatment under the protocol, and final results of the study will be available.

Chemotherapy of Pulmonary Photochromogenic Mycobacterial Infections in Man

LYNN C. CHRISTIANSON, PAUL L. RICHBURG, and HAL J. DEWLETT, Dallas, Texas

The photochromogenic mycobacteria are less susceptible than *M. tuberculosis* to the usual antituberculosis drugs both *in vitro* and in animals. A high percentage of initial drug resistance has also been observed in previously untreated patients with photochromogenic infections. These observations warranted a careful re-evaluation of the currently used chemotherapy in pulmonary photochromogenic disease.

Twenty patients with photochromogenic disease of the lungs have been treated with various combinations of the antituberculosis drugs. Results of therapy were assessed on the basis of sputum conversion, rate of cavity closure, other roentgenographic changes, and clinical course. Nine of the patients had surgical resections of residual lesions in addition to drug therapy, and tissue pathology and bacteriology were included in the results. Duration of observations ranged from 10 to 55 months, only 3 patients being followed for less than 12 months.

Observations thus far indicate: sputum conversion with and without chemotherapy, lack of correlation of sputum conversion with roentgenographic changes and/or tissue negativity, and progression of disease in 2 patients while under continuous drug therapy.

The Treatment of Tuberculosis of the Spine in Adults without Surgical Spine Fusion

BARRY FRIEDMAN, Cleveland, Ohio

Ten years ago a patient with tuberculosis of the spine refused an operation for fusion. She went on to heal her spinal lesion with bed rest and antituberculosis drugs alone.

Stimulated by this case, the author has now treated more than 60 patients without surgical fusion. The plan of treatment has been bed rest without casts or traction, antituberculosis drugs for prolonged periods, and ambulation with back braces after X-rays have shown no progression of disease.

The only surgical procedures carried out on these patients have been evacuation of large abscesses in some individuals and decompression laminectomies for paraplegia.

This represents a major departure from the classical treatment of spinal tuberculosis in adults, in which surgical fusion of the diseased segment has been considered an important feature.

The following conclusions have been drawn from this study:

Of 52 adults with tuberculosis of the spine followed for two years or more from the start of treatment, the spinal lesion has healed or has been controlled in every instance but one. Six patients in this series have died; in only one instance, however, could death be attributed to spinal disease, and in this case it was an operative cardiac arrest during costotransversectomy. An additional 10 patients with typical clinical and X-ray findings of tuberculosis of the spine but without bacteriologic proof have also been treated and have responded identically.

A variety of intervertebral bony bridges formed

spontaneously in many instances, giving good stability to the spine. Sinuses dried up and, in all but one case, remained closed. Abscesses not surgically drained have been absorbed or have become calcified.

Of 8 patients with paraplegia all but 2 have regained good function of their paralyzed extremities.

As might be expected, the response of the spinal disease in these individuals paralleled the response of their pulmonary disease when present.

The most important feature of treatment is the prolonged use of antituberculosis drugs.

The Management of Patients Excreting Typical Mycobacterium Tuberculosis Resistant to Both Streptomycin and Isoniazid

IRVING KASS, JAAKKO HAAPANEN, and WILLIAM F. RUSSELL, JR., Denver, Colorado

Between April, 1958, and May, 1959, a group of 54 patients excreting typical *Mycobacterium tuberculosis* resistant to streptomycin and isoniazid, who presumably had never received any of the secondary drugs (pyrazinamide, cycloserine, or kanamycin), were treated by random selection in an experimental program at National Jewish Hospital at Denver. Patients in treatment Group A received kanamycin, pyrazinamide, and cycloserine. Those in Group B received pyrazinamide and cycloserine. In addition, all received isoniazid and pyridoxine.

The dosage of pyrazinamide was 1.5 gm. daily, given in divided doses. The dosage of cycloserine was 0.5 gm. daily, given in divided doses. Between 4 and 5 gm. of kanamycin a week were given for a period no longer than five months.

The number of bacteriologic conversions obtained with medical treatment alone, plus the conversions that occurred in association with resections, occurred in 35 (65 per cent) of the 54 patients treated.

Nothing is to be gained by using the secondary drugs in piece-meal fashion; rather, therapy involving these drugs must be fashioned after the generally successful initial treatment program, in which combined and adequate therapy is the rule rather than the exception.

Effect of Withdrawal of Isoniazid in Chronic, Stable, Treatment-Failure Cases with Drug-resistant Tubercle Bacilli

EMANUEL WOLINSKY and VISHWA N. KAPUR, Cleveland, Ohio

Does it serve a useful purpose to continue the administration of isoniazid in the face of *in vitro* drug resistance and persistent failure to improve? A study was designed to answer this question. Thirty patients who had long-standing open pulmonary tuberculosis that had failed to respond to all therapeutic measures and who had relatively stable disease for the preceding year were selected. All medication was continued exactly as before except that similar-appearing placebo tablets were substituted for the isoniazid. Sputum cultures were obtained every month for isoniazid susceptibility and catalase activity. The study was continued for eight months or until a definite increase of disease occurred.

Of the 30 patients, 10 (one-third) showed unquestionable increases of disease within one to seven months. Seventeen patients exhibited no change clinically or by roentgenogram. There were 3 instances of disease increases which, in retrospect, had probably begun prior to the study. There was no significant difference in the percentage of worsening between the group of 21 patients whose bacilli were catalase negative prior to the study and the group of nine patients whose bacilli were catalase positive. In a few instances, increased catalase activity could be demonstrated in the serial cultures from patients whose bacilli were catalase negative previously.

It is concluded that the continued administration of isoniazid to the hopeless treatment-failure patient is warranted.

Streptomycin 0.5 Gm. Daily with Isoniazid and Para-Aminosalyclic Acid Used as a Re-treatment Regimen in Pulmonary Tuberculosis

WILLIAM S. SCHWARTZ and R. E. MOYER, Oteen, North Carolina

Beginning in April, 1958, the general use of a regimen consisting of 0.5 gm. of streptomycin, 300 mg. of isoniazid, and 12 gm. of PAS daily was begun in patients suffering from pulmonary tuberculosis who were in need of re-treatment. Indications usually consisted of failure to do well on a two-drug regimen or lack of information concerning drug susceptibility of tubercle bacilli in patients readmitted to the hospital. The regimen was begun in 212 cases; 151 patients have completed at least three months' treatment, 90 six months, and a few more than 12 months up to the present time.

Usual streptomycin toxicity was conspicuous by its absence; vertigo occurred in only 3 cases. Twenty-nine per cent of the patients were over 50 years of age and 15 per cent were over 60. The regimen was changed in 9 cases because of an increase of disease or lack of improvement. Other losses were due mainly to discharge of patients from the hospital. There were two deaths due to tuberculosis.

Patients with infectious sputum before treatment were divided into the eight groups possible,

according to the presence of organisms susceptible or resistant to streptomycin, isoniazid, and PAS. Resistance was defined for streptomycin and PAS as growth equal to the control in 10 γ per milliliter or any growth in 100 γ per milliliter. Corresponding levels for isoniazid were 1 and 5 γ per milliliter. When all cases were considered, the conversion rate was fair. When subdivided into groups, the conversion was excellent, with susceptibility to all drugs as might have been expected. It was fair when susceptibility to both streptomycin and PAS was present, poor with susceptibility only to streptomycin or PAS, and essentially nonexistent with resistance to all three drugs. Three other groups were too small for comment.

Cavities were present in 136 cases before treatment, 24 of which closed. This occurred nine times among the patients with susceptibility to all three drugs, three times among those susceptible to streptomycin and PAS, twice among those susceptible only to PAS, and ten times among those who had negative cultures prior to treatment.

Drug resistance rarely developed in infections initially susceptible to all three drugs. Resistance to one or two drugs developed commonly among those with susceptibility to two drugs, as well as when susceptibility to only one drug was present before treatment.

In conclusion, this triple-drug regimen was well tolerated but would seem to be useful only if bacterial susceptibility to at least two and preferably three drugs was present.

A Comparison of High-Dosage (16 Mg./Kg./Day) Isoniazid plus PAS and Low-Dosage (300 Mg./ Day) Isoniazid plus PAS in the Treatment of Cavitary Pulmonary Tuberculosis—Report of Veterans Administration–Armed Forces Study 19

H. William Harris, Salt Lake City, Utah, James A. Wier, Denver, Colorado, and Patrick B. Storey, Baltimore, Maryland

This study was designed (*1*) to compare the results in cases of cavitary pulmonary tuberculosis treated with high doses of isoniazid and PAS and those in cases treated with a lower dose of isoniazid and PAS, and (*2*) to investigate the possibility of a relationship between serum levels of biologically active isoniazid and the results of therapy with these regimens. Fifty-eight Veterans Administration and Armed Forces study unit hospitals participated in the study.

Previously untreated patients with bacteriologically proved, cavitary pulmonary tuberculosis were randomized between two therapeutic regimens. The high-dose regimen consisted of isoniazid, 16 mg. per kg. of body weight, given in three equally subdivided doses daily with PAS, 12 gm. daily, and pyridoxine. The low-dose regimen consisted of 300 mg. of isoniazid (100 mg. three times daily), and 12 gm. of PAS daily. The assigned regimen was continued for six months. Analysis of background factors (age, race, sex, trend of disease, extent of pulmonary involvement, number of cavities, and cavity size) indicated that the two groups were comparable. As of February, 1960, 314 patients were treated with the high-dose regimen and 326 patients with the low-dose regimen. The percentage of patients showing marked or moderate improvement, no change, and worsening of chest X-rays was almost identical in the two groups. The rates of bacteriologic conversion, cavity closure, and "target-point" were slightly greater in the high-dose regimen, although the differences were not statistically significant. There was no significant difference between regimens in the incidence of bacteriologic relapse, in the emergence of isoniazid-resistant strains, or in the persistence of isoniazid-susceptible strains. Toxicity to isoniazid occurred more often in patients given high doses of the drug.

An Evaluation of Chemotherapy in Pulmonary Tuberculosis—High Doses of Isoniazid plus PAS versus Low Doses of Isoniazid plus PAS

Stephen J. Berte and William G. Dunnington, Phoenixville, Pennsylvania

High doses of isoniazid plus PAS were given to 113 patients, and 76 currently tabulated patients received conventional doses of isoniazid plus PAS. After five to six months of treatment, 99 and 98.5 per cent of the patients had negative cultures, and cavities closed in 54.8 and 52.7 per cent of those in the high and low isoniazid treatment groups, respectively. Single- and multiple-cavity closure rates in the high versus the low isoniazid group were 63 versus 61.5 per cent, and 42.8 versus 44.8 per cent. Moderate to marked X-ray improvement occurred in 84.6 to 86.2 per cent of all cases. In summary, high-dosage isoniazid plus PAS did not appear superior to low-dosage isoniazid plus PAS in treating moderately and far advanced pulmonary tuberculosis.

2B Pulmonary Physiology

Hemodynamics of the Pulmonary Microcirculation

John W. Irwin, Walter S. Burrage, and Francis L. Weille, Boston, Massachusetts

A capacitance electromanometer was modified by placing the transducer at the end of a coaxial

cable. The transducer, plastic adapter, and microquartz cannula were combined into a unit that would fit onto a micromanipulator. The tip of the microcannula had a minimal diameter of 25 micra, and the microtip did not exceed 100 micra in length. The plastic adapter and microcannula were filled with a mixture of degassed Ringer's solution and heparin. Careful attention was paid to exclude air bubbles. The lung of a living anesthetized rabbit was exposed, and the microcirculation was observed with the aid of microscopes and transillumination. Then the tips of the microcannulae were inserted into pulmonary arterioles down to diameters of 50 micra, into pulmonary venules with diameters as small as 50 micra, into pulmonary arteries, and into the right ventricle of the heart. Dynamic pressuregrams were obtained. On occasion, simultaneous pressuregrams were obtained from the right ventricle and pulmonary arteriole or the pulmonary arteriole and pulmonary artery.

Pressuregrams obtained did vary in configuration, but there was no marked drop in systolic or diastolic pressure from the right ventricle to pulmonary arterioles of 50 micra. There appeared to be a more marked drop in pressure between pulmonary arterioles of 50 micra and pulmonary venules of 50 micra.

The Effect of Bronchospasm on Pulmonary Airway (Tracheal, Bronchial, and Postbronchial) and Circulatory Pressures—An Experimental Study

WATTS R. WEBB and LEONARD W. FABIAN, Jackson, Mississippi, JOSEF R. SMITH, Wood, Wisconsin, and G. D. CAMPBELL and M. A. CARNES, Jackson, Mississippi

This study uses two new techniques—one for producing bronchospasm experimentally and the other for measuring pressures distal to bronchospasm. A mecholyl spray introduced intratracheally in dogs will produce severe bronchospasm even to total ventilatory obstruction.

Measurements of "alveolar" pressures by interruption techniques are dependent upon pressure equilibrium between the alveoli and the airway, which may not occur in bronchospasm. By passing a small plastic catheter into the bronchiolar "wedge" position, tracings are obtained that reflect the postbronchiolar pressures.

In bronchospasm with spontaneous respiration, major airway pressures vary little, but tremendous swings occur in the pleural and postbronchiolar pressures. These are reflected in the circulation as measured by the pulmonary arterial wedge, pulmonary venous wedge, and pulmonary venous pressures.

With controlled respiration and bronchospasm, tracheal pressures follow the administered positive or negative pressure, but postbronchiolar and pleural pressures may show little or no change, demonstrating virtually complete obstruction in the small bronchioles. Pulmonary vascular dynamics are less affected, as the capillaries are also protected from the airway pressures.

Measurement of Gas Trapped in the Lung during Changing Airway Resistance

FRANK W. LOVEJOY, JR., HERBERT CONSTANTINE, JOSEPH FLATLEY, LUCIEN DAUTREBANDE, and NOLAN L. KALTREIDER, Rochester, New York

With the body plethysmograph to measure airway resistance and the volume of gas in the thorax, the helium-dilution method to measure the functional residual capacity, and the fast vital capacity tests, a study has been made of the effects of aerosols (either fine dust particles or carbachol) on respiration. Eleven normal subjects and 12 patients with chronic pulmonary disease were studied before and after the administration of constricting and dilating aerosols.

Inhalation of fine dust particles or carbachol uniformly caused an increase in airway resistance, which was accompanied by a significant reduction of the vital capacity and the mid-expiratory flow rate. A significant increase in the difference between the thoracic gas volume and the functional residual capacity was consistently demonstrated. Most of these effects occurred in normal subjects as well as in patients. Dilating aerosols always resulted in a return of all values to the control state, and the readministration of either dust or carbachol then usually failed to produce any change.

Since the thoracic gas volume as measured by the body plethysmograph includes all air in the thorax whether or not in communication with the trachea, an increase in this volume over that of the functional residual capacity indicates that constricting aerosols caused "air trapping" in both normal subjects and patients with chronic lung disease. In 8 other patients in whom a marked change in the airway resistance was produced, no consistent change in arterial blood gases was observed.

Application of Gas Chromatography to Determinations of Pulmonary Blood Flow and Diffusing Capacity

W. HAROLD LAWSON, JR., and ROBERT L. JOHNSON, JR., Dallas, Texas

Pulmonary capillary blood flow and diffusing capacity can be determined from changes in small

concentrations of respired carbon monoxide (CO) and acetylene (C_2H_2)—measured in respect to inert, insoluble sulfur hexafluoride (SF_6)—that have occurred during breath-holding. To analyze these gases in one sample, we adapted the Beckman GC-2 chromatograph. Oxygen, N_2, and CO_2 can also be analyzed.

The unknown mixture passes in a helium stream through silica gel and molecular sieve adsorbents, where the gases are separated, and the separated components pass through a thermal conductivity cell that yields a recorded signal. Acetylene or CO_2 inactivates the molecular sieve; thus they are shunted past this material with a special valve.

The relationship between area under the recorded signal and concentration for each gas is linear. Sensitivity exceeds 0.01 per cent; CO and reproducibility is within 1 per cent. Total analysis time is 18 minutes.

Ventilation-Perfusion Ratio Determination Using a Gas Partitioner

H. G. Boren and F. L. Kracke, Houston, Texas

Previous methods of ventilation-perfusion ratio measurements have been intricate or have required expensive equipment. As each component of a respiratory gas mixture was analyzed independently by the gas partitioner, it was used to measure variation of expired gas-exchange ratio with a high degree of precision. A simple means was devised to obtain samples after 750 and 1,250 ml. of expiration. Analysis of these samples with the partitioner allowed calculation of expired gas exchange and, thereby, an estimate of evenness of ventilation-perfusion ratios. All that was required of the patient was a single expired breath. The complete determination was done in less than 15 minutes. The addition of inert gas to the inspired breath or inspiration of 100 per cent oxygen allowed a separate estimate of distribution of air. Uneven distribution of air was then compared with uneven distribution of ventilation-perfusion ratio. Differences between normal persons and those with various pulmonary diseases were well demonstrated.

A Simple and Rapid Method of Determining Pulmonary Diffusing Capacity by the Carbon Monoxide Steady-State Method

Stanford K. Sweany, Chicago, Illinois, John T. Sharp, Hines, Illinois, and George A. Saxton, Jr., Chicago, Illinois

A simple, rapid, and accurate estimation of the alveolar carbon dioxide tension by a "rebreath technique" has been applied to the steady-state measurement of the carbon monoxide diffusing capacity. During rebreathing of the appropriate CO_2 mixture, alveolar and mixed venous arterial pressure of the carbon dioxide (pCO_2) attain equilibrium. The arterial pCO_2 can be estimated by subtracting 6 mm. Hg., the average normal arteriovenous (A-V) difference in pCO_2. Forty serial measurements of the D_{CO} by this method over a two-year period in one normal subject and in 4 patients with stable diffusion defects have demonstrated a maximal difference of 2.3 ml./min./mm. Hg. on consecutive evaluations.

In patients with both reduced cardiac output and impaired ventilation, the A-V difference in pCO_2 was greater than 6 mm. Hg. An accurate estimate of arterial pCO_2 by this method cannot be obtained in these cases.

Advantages of the method are: avoidance of arterial puncture, consistent and accurate estimation of arterial pCO_2 in most cases, and rapidity, involving an average time of 15 minutes for the determination of D_{CO}, including gas analyses and calculations.

An Appraisal of the Breath-holding Method for Measuring D_{CO} in Patients with Nonuniformity of Alveolar Gas and a Simplification of the Measurement of Alveolar Volume

Nola Mae Moore, Theodore C. Smith, Alice L. Castillo, and John Rankin, Madison, Wisconsin

Breath-holding techniques for measuring diffusing capacity (D_{CO}) are frequently challenged with the following questions:

Is the air sample measured truly representative of the lung as a whole?

Can you measure the alveolar volume from which diffusion takes place?

Does carbon monoxide (pCO) fall exponentially in relation to time?

Three independent measurements of D_{CO} were made in 20 normal adults and 90 emphysematous patients with demonstrable abnormality in distribution of inspired gas. Alveolar volume in each case was determined by the seven-minute washout technique of Darling, and independently calculated from the helium dilution. We found no significant differences between D_{CO} values based on alveolar volumes so measured. The time of breath holding was varied from 10 to 20 seconds in 50 patients and was constant at approximately 10 seconds in 40 patients. Despite 100 per cent variation in time, the calculated D_{CO} did not vary significantly. The standard error of mean values obtained for D_{CO} in the emphysema groups compared favorably with similar values for normal adults.

We conclude that F_{ECO}/F_{ICO} falls exponentially

up to 20 seconds in patients with emphysema, that nonuniformity of alveolar gas does not significantly influence the reproducibility of D_{CO} by this technique, and that the dilution of helium may be used to measure alveolar volume, hence simplifying the method.

Ventilation, Dead Space, and Hemoglobin Level as Modifiers of the Steady-State Method for Diffusing Capacity for Carbon Monoxide

KAYE H. KILBURN, Denver, Colorado

In experiments to evaluate the extent to which rate, volume, and pattern of ventilation, the physiologic dead space, and hemoglobin level alter the carbon monoxide diffusing capacity measured during the steady state (D_{CO}), these parameters were varied individually in a series of dogs. D_{CO} measurements were made in 7 spontaneously ventilating and 22 apneic dogs ventilated with a pump. The D_{CO} and fractional uptake of CO (F_{CO}) were measured at (*1*) various ventilatory rates with a constant stroke volume, (*2*) constant rate and various volumes, (*3*) constant rate and volume with various dead spaces, and (*4*) constant ventilation and blood volume with stepwise reduction of hemoglobin. Respired air and arterial blood were analyzed by conventional methods, and cardiac outputs measured by the Fick principle. Expired air CO levels were measured on an infrared analyzer.

Dogs ventilating spontaneously had a mean D_{CO} (ml./min./mm. Hg.) of 9.5 with a minute ventilation (MV) of 2.2 L., a D_{CO} of 6.5 with an MV of 2.6 L. at one hour, a D_{CO} of 9.2 with an MV of 3.6 L. at three hours, and a D_{CO} of 10.2 with an MV of 4.5 L. at six hours. When tidal volume was 300 ml. and ventilatory rate was increased from 10 to 23/min. in 5 dogs, D_{CO} was 11.5 at 10, 10.6 at 14, 17.1 at 17, 9.5 at 20, and 10.6 at 23. Increments of 25 ml. in external dead space decreased D_{CO} from 35.9 at 0 to 19.7 with 25 ml., 25.1 with 50 ml., 12.9 with 75 ml., and 12.5 with 100 ml. Serial 20 per cent reductions in hemoglobin to one-fifth of initial reduced D_{CO} from 33.2 to 14.8, 12.7, 14.2, and 6.7; cardiac output increased from a control of 1.76 L. to 4.26 L. at a hematocrit of 16 mm. but fell to 3.44 L. at a hematocrit of 9.7 mm.

Carbon monoxide diffusing capacity determinations reached a maximum at 17 resp./min. in dogs ventilated at 310 ml./stroke. Increased external dead space decreased D_{CO}. Reductions in hemoglobin reduced D_{CO} and raised cardiac output. The quantity measured as D_{CO} cannot be divorced from ventilation, dead space, and hemoglobin level. These influences should be considered in evaluating the steady-state D_{CO}.

4A Pulmonary Physiology

Pathophysiologic Features of Pulmonary Alveolar Proteinosis with Notes on Differential Diagnosis—A Report of Six Cases

EDWARD A. GAENSLER, Boston, Massachusetts, ASHER MARKS, Miami, Florida, and EUGENE D. ROBIN, Pittsburgh, Pennsylvania

In 1948, Rosen, Castleman, and Liebow published a report on 27 cases presenting a remarkable lung picture, which consisted of ". . . deposition of a PAS-positive granular and floccular proteinaceous material, rich in lipids, within the lumens of the distal air spaces, where little, if any, reaction is usually produced."

Two of these patients were seen at our laboratories some years before and have been followed at intervals since. One died as the result of his disease, and the other recovered completely. We have performed extensive respiratory physiologic studies on 4 other patients with this "disease." Pathologic diagnoses were made by lung biopsy in 4, by autopsy in one, and by both pre-mortem and post-mortem examination of lung tissue in one.

The present report deals with the respiratory studies in these 6 cases. The pathophysiology early in the disease was one of "pure" oxygen diffusion impairment with supernormal breathing capacities, slightly and symmetrically reduced lung volumes, grossly normal distribution of gas, severe alveolar hyperventilation and respiratory alkalosis, alveolar-arterial gradients up to 90 mm. Hg., and hypoxemia during exercise. At a later stage of the disease there was more restriction of lung volume, hypoxemia at rest with oxygen saturations as low as 30 per cent, the lowest diffusing capacities ever reported in adults, cor pulmonale, polycythemia, and survival only on continuous oxygen therapy.

The nature of the "alveolar-capillary block," the role of shunt hypoxemia, the possibility of alveolar-arterial carbon dioxide gradients, and possible etiologic factors of this disorder are all to be considered.

Unilateral Hypoventilation during Temporary Ipsilateral Pulmonary Artery Occlusion in Man

E. W. SWENSON, T. N. FINLEY, S. V. GUZMAN, and J. W. SEVERINGHAUS, San Francisco, California

Fourteen patients with chronic lung disease have been studied by means of temporary unilateral pulmonary arterial occlusion, using a triple-lumen cardiac catheter, with a thin-walled latex balloon mounted over a middle orifice near

the tip of the catheter. Following a one-sided pulmonary arterial occlusion, the total ventilation increased an average of only 10 per cent, even though a normal arterial carbon dioxide tension indicated that alveolar hypoventilation had not occurred. This was studied further in 11 cases by means of bronchospirometry during the occlusion. Obstructing the pulmonary arterial flow to one lung was followed by a decrease in the proportion of the total ventilation performed by the lung in which the supply of mixed venous blood was blocked (mean before occlusion 45 per cent, during occlusion 31 per cent). The anatomic dead space of the bronchial tree on that side diminished from a mean of 38 ml. before to 23 ml. during occlusion. Bronchograms showed that the balloon-filled segment of the pulmonary artery did not impinge on its neighboring bronchus. It has been possible to block this hypoventilatory response by giving the lung 6 per cent CO_2 to breathe during the blockade. Our results suggest that bronchoconstriction occurs in response to the lowering of airway CO_2 tension and is a mechanism whereby an "unperfused" lung can diminish the "alveolar dead space" that would otherwise result.

The Effect of Hyperventilation and of Bronchodilator Drugs on Alveolar Ventilation in Patients with Chronic Obstructive Pulmonary Emphysema

AARON A. COHEN, San Fernando, California, and ALLAN HEMINGWAY, Los Angeles, California

The displacement of N_2 from the alveolar spaces of emphysematous lungs during O_2 breathing is markedly delayed. The questions investigated in this study of 12 patients with emphysema were (*1*) whether more N_2 clearance occurred for equivalent volumes of O_2 passing in and out of the lungs during intermittent positive pressure breathing with and without isoproterenol (IPPB ± ISP) than during ambient pressure breathing (APB), and (*2*) whether aminophylline facilitated N_2 clearance.

Aminophylline had no significant effect on N_2 clearance. Hyperventilation produced by IPPB alone and by IPPB + ISP led to lower N_2 concentration in the poorly ventilated spaces that are uncovered late in the course of the washout procedure. The improvement in ventilation of the "slow spaces" was produced by less O_2 during hyperventilation than during APB. A significant effect on the N_2 clearance from the better ventilated spaces was not apparent but may have been masked by a greater contribution from the less ventilated spaces during the earlier phases of the N_2 washout procedure. The effect was greater with IPPB + ISP than with IPPB alone. In the group as a whole the improvement in N_2 clearance, in terms of effect produced by comparable volumes of O_2, was not striking, but in one individual it was marked.

Pulmonary Diffusing Capacity in Resting Subjects with Normal and Increased Pulmonary Blood Flow

GEORGE N. BEDELL, Iowa City, Iowa

This study was undertaken to discover what functional changes occur in the lungs of resting persons who have increased pulmonary blood flow —patients with atrial or ventricular septal defects. Patients with normal pulmonary arterial pressure were deliberately chosen to avoid including patients with extensive pulmonary vascular disease.

Ten normal subjects and 20 subjects with either atrial or ventricular septal defects were studied. There were no significant differences between the normal subjects and the patients with increased pulmonary blood flow in the measurements of lung volumes, minute volume of ventilation, distribution of inspired air, and maximal breathing capacity. The mean diffusing capacity of the lung for carbon monoxide (D_{LCO}) was 31 ml./mm.,Hg./min. or 106 per cent of predicted normal in normal subjects; and in patients with increased pulmonary blood flow, D_{LCO} was 35 ml./mm.Hg./min., or 140 per cent of predicted normal. The groups are significantly different ($P = <0.01$). A plot of D_{LCO} against pulmonary blood flow shows a tendency for D_{LCO} to be greater as flow increases.

The Effects of ThoracicSurgery on the Pulmonary Diffusing Capacity

FRANCES R. DIETIKER, Hinsdale, Illinois and BENJAMIN BURROWS, Chicago, Illinois

Carbon monoxide breath-holding diffusing capacities (D_L) have been determined for 68 patients before and approximately four weeks after various types of pulmonary surgery. Nine pneumonectomy patients showed a 46 per cent fall in both D_L and lung volume (V_L). After lobectomy, 34 patients showed slightly more loss of D_L than of V_L (28 per cent and 25 per cent, respectively). Following thoracoplasty, wedge resection, or segmental resection, however, D_L decreased considerably more than the V_L. Twelve patients showed an average D_L loss of 30 per cent and a V_L loss of 19 per cent following extraperiosteal paraffin plombage, and 13 patients undergoing wedge or segmental resection showed an average D_L loss of 22 per cent and V_L loss of 14 per cent. When some of the patients were subsequently retested, four weeks to one year after the first

postoperative test, both D_L and V_L showed improvement.

It is concluded that pulmonary surgery of lesser extent than pneumonectomy produces a temporary diffusion loss that cannot be explained by loss of lung volume alone and indicates an alteration in pulmonary physiology.

The Relationships of Hypercapnia to Thyroid Function

MYRON STEIN, DAVID SINGER, and MILTON W. HAMOLSKY, Boston, Massachusetts

The clinical resemblance of hypercapneic patients to hyperthyroid subjects is often striking. Recent reports indicate therapeutic benefit in such patients following the induction of myxedema by I^{131}, although there was no significant change in pulmonary function studies. Moreover, measurements of serum-protein-bound iodine and thyroidal radioactive iodine uptake have usually been normal in hypercapneic patients. In an investigation of the possible relationships of hypercapnia to thyroid function, multiple pulmonary and thyroid function tests were performed on 15 hypercapneic subjects. Pulmonary studies included lung volumes, maximal flow rates, distribution of gas, maximal breathing capacity (MBC), and alveolar pCO_2 measured on arterial blood by conventional methods and/or end-expired (alveolar) air with a pCO_2 meter. The thyroid tests included protein-bound iodine (PBI), 24-hour thyroidal I^{131} uptake, turnover of infused I^{131} thyroxin, and a newly devised *in vitro* test that measures plasma-protein binding and tissue distribution of labeled thyroid hormones and has been applied to more than 5,000 patients in our laboratories.

In 11 hypercapneic male patients, the erythrocyte uptake ranged from 17.8 to 35.8 per cent, and averaged 21.5 per cent. In 4 hypercapneic females, the uptake ranged from 20.6 to 32.9 per cent, and averaged 25 per cent. These levels of erythrocyte uptake are within the hyperthyroid range; normal values in males range from 11.5 to 18.5 per cent; in females, from 11 to 17 per cent. In 6 patients with pulmonary emphysema and normal alveolar pCO_2, the erythrocyte uptake was within normal limits. In 3 patients with hypercapnia in whom remission of disease resulted in decrease of alveolar pCO_2, there was also a decrease in erythrocyte uptake from elevated toward normal levels.

In addition, thyroxin I^{131} turnover was measured in 8 subjects with hypercapnia and was accelerated in all patients. Half-times of disappearances ranged from 3 to 5.8 days with an average of 4.8 days; normal euthyroid half-life is from 6.8 to 7 days.

The abnormalities observed cannot be related to pH changes alone, as several of the patients had hypercapnia with normal arterial blood pH. These studies, suggesting an abnormality of peripheral utilization of thyroid hormone in hypercapnia, may explain improvement of hypercapneic patients following treatment with myxedema-inducing doses of radioactive iodine.

The Effect of Isoproterenol on the Heart and Pulmonary Circulation in Patients with Chronic Pulmonary Emphysema

JOHN F. WILLIAMS, DOUGLAS H. WHITE, and ROY H. BEHNKE, Indianapolis, Indiana

It has been stated that patients with chronic lung disease cannot decrease their pulmonary vascular resistance in response to pharmacologically induced increase in cardiac output, and that isoproterenol raises pulmonary arterial pressure as it increases cardiac output. Observations on 9 patients with chronic pulmonary emphysema without congestive heart failure in whom isoproterenol had been infused directly into the pulmonary artery have not substantiated these statements.

Right heart catheterization and routine pulmonary function studies were performed in all 9 patients. Pulmonary and systemic arterial pressures were determined in all at rest and during the infusion of 0.005–0.010 mg./min. of isoproterenol, also in 7 patients during steady-state exercise and during exercise with drug infusion in similar amounts. Cardiac output was determined under these conditions in all but one patient by the direct Fick principle, and "wedge" pressures were determined when technically possible.

The resting mean pulmonary arterial pressure (range 13–40 mm. Hg.) was elevated in 5 patients, and the infused drug effected a significant drop in 2 (8 and 15 mm. Hg.). The resting cardiac output with infusion increased in 4 patients (778–4873 cc./min.), was unchanged in 2, and decreased in one. Pulmonary vascular resistance was decreased in all 5 in whom it was determined.

With exercise, 5 of 7 patients developed pulmonary hypertension. In 4 the drug reduced the pressure, and the drop was found to be generally proportional to the severity of the hypertension (e.g., 54-26, 46-35 mm. Hg.). Cardiac output during steady-state exercise with isoproterenol as compared with that of exercise alone was increased in 5 of 6 patients (660–2715 cc./min.). During exercise the drug reduced the pulmonary vascular resistance.

In no portion of the study did the oxygen consumption during drug infusion, as compared with its parallel control state, increase enough to account for the increased cardiac output.

Patients with chronic pulmonary emphysema can decrease their pulmonary vascular resistance and pulmonary arterial pressure dramatically as they increase their cardiac output during isoproterenol administration. It is suggested from these studies that there is a definite element of active pulmonary vasoconstriction contributing to the hypertension in these patients, and that this is diminished by the infusion of the drug. Also, an increase in cardiac output cannot be interpreted as indicating left heart failure in such patients.

4B Experimental Chemotherapy

A Simple Test to Distinguish Rapid and Slow Inactivators of Isoniazid

H. William Harris, Ralph A. Knight, Dorian R. Faber, and Merle L. Selin, Salt Lake City, Utah

Previous studies in this laboratory indicated that the rate of isoniazid inactivation in humans is genetically determined. "Rapid" inactivation is inherited as a dominant trait and "slow" inactivation as a recessive trait. Although the six-hour serum isoniazid concentration, as measured in this laboratory, will segregate groups of individuals into rapid and slow inactivators, it will not distinguished heterozygous from homozygous genotypes.

The quantity of "free" isoniazid excreted in the urine depends upon the serum concentrations of the free drug. However, the urine concentration is 10 to 100 times greater than the serum concentration and may be measured by chemical methods. Attempting to quantitate accurately the extent of *in vivo* inactivation, we measured the urinary excretion of free isoniazid after a 4 mg./kg. dose in 72 subjects.

A distribution curve that plots the urinary free-isoniazid excretion, expressed as a percentage of dose, among these subjects reveals bimodality, and the subjects can be classified as rapid or slow inactivators.

Since it is difficult to collect 24-hour urine specimens, particularly in large groups of subjects as required for genetic investigation, a simple modification of this test was devised. The subjects were given 4 mg./kg. of isoniazid at 10:00 p.m., and the urine was collected for a ten-hour period, until 8:00 a.m. The quantity of free isoniazid in the ten-hour urine specimen (expressed as a percentage of dose) is compared with the classification provided by the six-hour serum concentration in these subjects.

Except for one individual, this simple test segregated rapid and slow inactivators of isoniazid identically with that provided by the six-hour serum classification. A test based on collection of an overnight urine specimen has definite practical advantages as compared with the serum isoniazid test. This method of segregating individuals into rapid and slow inactivators of isoniazid may be of great value in studying large population groups.

Effect of Cortisone plus Antituberculosis Agents on Tuberculous Pneumonia in Guinea Pigs—Preliminary Report

Alfred G. Karlson and David T. Carr, Rochester, Minnesota

Forty guinea pigs received intravenous injections of 0.1 mg. of tubercle bacilli. Eight days later, they were divided equally into four groups, with treatment given subcutaneously once daily as follows: group 1, untreated controls; group 2, 2 mg. of cortisone; group 3, 2 mg. of streptomycin; group 4, 2 mg. of cortisone plus 2 mg. of streptomycin in one mixture.

The controls and those given cortisone alone were dead 28 days after the injection of the tubercle bacilli. Three given streptomycin alone and 6 given cortisone plus streptomycin survived; all of them were killed on the twentieth day of treatment. Tuberculous pneumonia was found in all of the animals, but it was less extensive in those given combined treatment. Histologic grading of the severity of the lesions from 1 to 4 gave the following averages: controls, 3.8; those given cortisone, 4; those given streptomycin, 3.8; those given cortisone plus streptomycin, 1.8.

Studies on the effect of cortisone and isoniazid in various amounts are incomplete, but enough data are available to permit some conclusions. For example, 40 guinea pigs infected as above were divided seven days later into groups of 10 animals each, with the following treatment: group 1, untreated controls; group 2, 5 mg. of cortisone; group 3, 0.2 mg. of isoniazid; group 4, 5 mg. of cortisone plus 0.2 mg. of isoniazid. The controls, those given cortisone alone, and those given isoniazid alone were all dead in 23, 25, and 26 days, respectively. Those given combined treatment had a longer survival time, 4 living on the twenty-eighth day. Histologically, the lesions in this last group were less severe.

It is concluded that the administration of cortisone plus either streptomycin or isoniazid in proper doses to guinea pigs with tuberculous pneumonia prolongs the survival time and limits the spread of the disease.

The Chemotherapy of Experimental Pulmonary Tuberculosis: Correlation between Survival of Rabbits and the in Vitro Demonstration of Viable Tubercle Bacilli

Sidney Bernstein and Nicholas D. D'Esopo, West Haven, Connecticut

The correlation was investigated between survival of animals and the *in vitro* demonstration

of viable tubercle bacilli from residual pulmonary lesions in rabbits after prolonged chemotherapy. A total of 72 rabbits was exposed to infective aerosols of bovine tubercle bacilli. Six weeks after infection, all animals were X-rayed. Six animals were sacrificed, and the number, distribution, and size of the pulmonary lesions were determined. The remaining rabbits received daily single intramuscular injections of 100 mg. of streptomycin and 24 mg. of isoniazid (Pfizer's Streptohydrazid®) for one year without interruption.

After one year of continuous drug therapy, 51 rabbits survived. At that time, these were divided at random into two groups of 26 and 25 animals.

One group of 25 rabbits was sacrificed. The size, number, and morphology of the residual lesions were determined, and 71 specimens were selected for bacteriologic study. Several media, including one permitting prolonged incubation, and animal inoculation were used for viability studies. Positive smears for acid-fast bacilli were obtained from 48 (68 per cent) of the specimens, whereas only 2 (3 per cent) yielded cultures positive for tubercle bacilli.

The group of 26 rabbits received no further chemotherapy and served as controls. Twenty-four months after the cessation of chemotherapy, 13 animals were alive and without evidence of active tuberculosis, as revealed by X-rays and weight records. Four rabbits died of progressive tuberculosis (3 of these relapses were shown by X-ray within four months), one each died during the eighth and ninth months, and 2 during the tenth month after therapy was discontinued. Nine animals died of nontuberculous causes; these deaths occurred in a purely random manner.

These results show that the *in vitro* demonstration of the viability of tubercle bacilli in the rabbit after prolonged chemotherapy corresponds closely to the pathogenicity of these organisms in residual necrotic lesions.

Correlation of Drug Susceptibility and Metabolic Activity of Various Strains of Mycobacteria

D. Koch-Weser and R. P. Palmer,
Cleveland, Ohio

Lack of susceptibility to antituberculosis drugs has become a clinical problem in infections with anonymous mycobacteria. Therefore, the possible relationship between susceptibility to drugs and metabolic activity is of practical and theoretic interest.

Measurement of metabolic activity was done by determining growth in liquid medium by nephelometric readings; triphenyltetrazolium reduction, as an indication of dehydrogenase activity; C^{14}-labeled acetate utilization in Conway units, separating the uptake of C^{14} into the bacillary substance from the $C^{14}O_2$ produced as an indicator of energy expenditure.

The ten investigated strains were H37Ra and R1Rv (two laboratory strains closely related to virulent human tubercle bacilli, H37Rv, and identical with this virulent strain in both relatively slow growth and metabolism), two strains of photochromogens, two strains of scotochromogens, two strains of nonpigmented anonymous mycobacteria (all of these six strains grew much faster and also exhibited more dehydrogenase activity and acetate utilization), *M. smegmatis*, and *M. phlei* (very rapidly growing and intensely metabolizing saprophytic strains).

Tested for their effect on growth and metabolic activity of these strains *in vitro* were isoniazid, streptomycin, neomycin, and kanamycin. They were added to the medium in increasing (tenfold) concentrations.

C^{14}-labeled isoniazid was added to each of the ten strains during the logarithmic growth phase and after 48 hours the amount of the drug "taken up" or "bound" was determined.

With increasing growth and metabolic activity, the inhibiting concentration of all four tested drugs also increases, the human strain being inhibited by the smallest, the saprophytes by the largest (for instance, 200 γ of isoniazid/ml.) concentrations.

There is no specific susceptibility of the anonymous strains to neomycin and kanamycin.

Neomycin and kanamycin in the same concentrations exhibit almost identical potency in every experiment. They are very closely related.

The binding of isoniazid is determined not by the metabolic activity but by the mass of bacterial substance. With 10 γ of C^{14} isoniazid/ml., human strains and saprophytes take up the same amount of drug or its derivative per nephelometer unit, the former one being inhibited, the latter one not. This lends support to our previously expressed hypothesis that the initial binding of the drug is an adsorption phenomenon that has to be followed by metabolic utilization and, therewith, inhibition.

A Rapid Method of Testing the Susceptibility of Mycobacteria to Antituberculosis Drugs

C. O. Rogers, I. G. Melvin, and H. S. Willis,
Chapel Hill, North Carolina

Accepted techniques of susceptibility testing in the field of tuberculosis tend to be tedious, time-consuming, and often inaccurate when one considers preparation of media and incubation time. When the results of susceptibility tests must be had before a clinician begins a chemotherapy regimen, decreasing the time involved becomes important.

The total number of living cells present in suspensions of normally metabolizing cells can be standardized on the basis of milligrams of red formazan reduced from 2,3,5 triphenyltetrazolium chloride (TTC). It seems logical, therefore, that inhibition of normal metabolic activity of the cells would result in decreased reduction of the TTC.

Cultures isolated on Löwenstein medium from patients were ground in Dubos basal medium, large clumps removed by low-speed centrifugation, and equal aliquots of the suspension exposed to varying concentrations of streptomycin and isoniazid hydrazid for four days at 37° C. The cells were washed free of the drug and incubated with TTC. Cells remaining metabolically active reduced TTC to formazan (as they did prior to drug exposure), the concentration of which was determined colorimetrically.

Cells susceptible to the drugs failed to reduce the TTC, whereas resistant cells retained the capacity to reduce it. Mixed populations of susceptible and resistant cells or partially resistant organisms demonstrated decreased capacity for reduction as compared with the capacity of controls not exposed to the drugs, and drug-enhanced organisms demonstrated even greater reduction than the controls.

This method of susceptibility testing compared favorably with the media culture technique and reduced the time required for susceptibility testing from two weeks to four days.

A Rapid Drug-Susceptibility Test for Acid-fast Bacilli

G. P. Kubica, Chamblee, Georgia

The disadvantage of most drug-susceptibility tests is the length of time required for performance and reading of results. After the tubercle bacilli have been isolated, it usually takes two or three weeks to determine their susceptibility. The present technique describes modifications of procedures by Sorensen, Pital, *et al.*; in the present technique, culture viability is indicated through use of an oxidation-reduction indicator, resazurin.

Known concentrations of various antituberculosis agents are prepared either by serial dilution of stock solutions of drug or by the addition of Bacto sensitivity discs to 1 ml. of Tween®-albumin broth containing the organisms. The inoculum of organisms is prepared by diluting (10^{-1} or 10^{-2}) in Tween-albumin broth a seven- to ten-day-old broth culture or a saline suspension of the organisms whose antimicrobial spectrum is desired. One milliliter of this inoculum is added to each tube containing either a drug disc or 0.1 ml. of the appropriate stock drug dilution in water; a nondrug-containing tube is inoculated as a control. Inoculated tubes are placed in the incubator for two to four days. Then one drop of 0.05 per cent resazurin is added to all tubes, and the tubes are put back in the incubator. When the control tubes turn pink, indicating active bacillary metabolism, the drug tubes are observed; a pink to red coloration indicates no inhibition by the drug: a blue shows complete inhibition; and a purple suggests partial inhibition of organisms. Comparison of this technique with a routine drug-susceptibility test using solid media showed excellent correlative results.

4C Fungous Diseases

Amphotericin-B Therapy of Histoplasmosis

W. D. Sutliff, Memphis, Tennessee

The therapeutic effects of amphotericin-B in histoplasmosis vary with the clinical type of the disease. It is reported to be an effective agent for the disseminated form of histoplasmosis. It has been used only in cases of unusual severity in the acute pulmonary form, and few reports are available. In the chronic pulmonary form of histoplasmosis, therapeutic effects were limited by the persistence of chronic lesions and by the toxicity of amphotericin-B.

A cooperative, randomized study of amphotericin-B therapy of histoplasmosis was, therefore, undertaken in Veterans Administration and Armed Forces hospitals. A series of 19 cases of chronic pulmonary histoplasmosis randomized to compare modified hospital rest therapy with amphotericin-B administration were studied. Five courses of amphotericin-B therapy were also given without randomization. Prompt conversion of the sputum was regularly observed. Definite but limited changes occurred in pulmonary lesions. Subjective improvement occurred with both regimens but more often after amphotericin-B. Bacteriologic relapses occasionally occurred, and these cases responded to re-treatment. Toxic reactions were frequent and prevented successful therapy in some instances.

It is concluded that amphotericin-B therapy is effective and is the therapeutic agent of choice, but that it has definite limitations, and more effective and less toxic therapeutic agents should continue to be sought.

Observations on Experimental Histoplasmosis in Dogs

John J. Procknow, Evelyn Saxon, and C. George Ray, Chicago, Illinois

Experimental histoplasmosis has been induced and closely observed up to four years in dogs

exposed to air-borne *H. capsulatum* spores, using a simple closed system simulating the natural route of infection via the respiratory tract. Infection of 36 nonimmunized and 8 immunized dogs produced the complete spectrum of disease, ranging from unapparent to disseminated histoplasmosis. Seventeen dogs have been reinfected up to seven times; 8 were repeatedly infected by bronchoscopic placement of a spore suspension into the right lower lobe.

Serial determinations were made of each animal's temperature, weight, blood count, sedimentation rate, hematocrit, skin tests, complement-fixation and precipitin antibodies, and blood and bone marrow cultures. Pulmonary involvement was frequently observed roentgenographically.

One dog succumbed to disseminated histoplasmosis. The majority became infected and showed a remarkable tendency to resolution and healing. Antibody levels persist for years. Resistance to reinfection with air-borne spores was marked, and pulmonary lesions were minimal. Necrosis and cavitation did not occur following multiple reinfection with air-borne or localized bronchoscopic placement of spores.

The Serious Prognosis of Untreated Histoplasmosis

J. Lewis Yates, Mount Vernon, Missouri

In the follow-up studies of 250 cases of proved histoplasmosis, the diagnosis was established or confirmed at this station. All cases were followed up six months later, with extremely interesting results. Almost a quarter of the patients with proved histoplasmosis had died in the subsequent period of follow-up. Death rates differ markedly for different types of disease and for different age groups. These studies form the basic controls for therapeutic studies of histoplasmosis.

High Incidence of Histoplasmosis in Sanatoriums in the United States

Michael L. Furcolow, Kansas City, Kansas, Joseph H. Schubert, Chamblee, Georgia, and Fred E. Tosh, Kansas City, Kansas

This study reports the results of serologic and pathological studies in tuberculosis sanatoriums throughout the United States, particularly the central United States. These studies have included more than 25,000 patients, and the original 1957 estimate of 1,200 cases of probable histoplasmosis in the tuberculosis sanatoriums in the United States has been exceeded. These studies have shown a high incidence of positive serologic tests and of positive cases of histoplasmosis by culture or pathologic study in sanatoriums in the United States, particularly in the central part of the United States. Indeed, estimates based on the survey as presently reported indicate that there are probably between 3,000 and 4,000 cases of histoplasmosis in the sanatoriums in the United States.

Nocardiosis—An Analysis of Nine Cases

John F. Murray and Sidney M. Finegold, Los Angeles, California, Seymour Froman, Olive View, California, and Drake W. Will, Los Angeles, California

Nine cases of nocardiosis, proved by cultural techniques in 8 patients and characteristic staining reactions and smears in one, have been encountered in the past seven years. Perhaps the most notable characteristic of nocardiosis was the wide clinical spectrum encountered. Ages ranged from 4 to 91 years in patients who were initially diagnosed as having dysgammaglobulinemia, acute pneumonia, active and chronic tuberculosis, purulent meningitis, bronchogenic carcinoma, or pemphigus.

Types of nocardiosis seen were a localized granuloma of the lung (one case), chronic progressive fibrosis of the lung resembling fibronodular tuberculosis (one case), acute bronchopneumonia, often with empyema and/or chest wall extension (4 cases), and a generalized form with involvement of the lungs, pleura, chest wall, brain, spleen, kidneys, and pericardium (3 cases). Possible predisposing factors to nocardial infection were prolonged high-dosage steroid or antimicrobiol therapy, malnutrition, diabetes mellitus, and an altered immune mechanism.

Although nocardiosis was not considered in the initial diagnosis of any patient, the diagnosis was eventually made by recovery of the organism from sputum, thoracentesis fluid, or abscess drainage prior to death in 6 patients. In one case the disease was unsuspected until a resected lung specimen was cultured and examined microscopically. Five of the 9 patients died, a mortality rate of 56 per cent. Three of these patients died in spite of proper ante-mortem diagnosis and appropriate antibacterial chemotherapy; in the other 2 fatal cases the disease was not recognized until an autopsy was performed. The 4 living patients were treated with a combination of sulfonamides and other antimicrobial agents and surgical resection or drainage. The sputum of one patient has again become infectious and he is being treated.

Requisites for improving survival in this serious disorder are awareness of the potentialities of nocardial infection, early diagnosis, and adequate medical and surgical therapy.

5A Surgery

Early Resection for Pulmonary Tuberculosis

RICHARD F. KIEFFER, JR., PATRICK B. STOREY, and DONALD H. HOOKER, Baltimore, Maryland

Since 1953, a number of patients at the Veterans Administration Hospital in Baltimore have undergone resectional surgery for pulmonary tuberculosis after having received antituberculosis chemotherapy for less than the usual period of time. Approximately 45 of these patients had demonstrable cavities and infectious sputum at the onset of treatment, were initially treated patients, and were brought to surgery less than 90 days after the initiation of antituberculosis chemotherapy.

As a result of their experience with these cases, the authors have considered whether pulmonary resectional surgery performed after a relatively short course of antituberculosis chemotherapy is associated with an increase in tuberculous complications or technical difficulties.

The Effects of Extraperiosteal Thoracoplasty with Paraffin Plombage on Pulmonary Function

GORDON L. SNIDER, ALLAN R. SHAW, and WILLIAM M. LEES, Chicago, Illinois

Ninety-three patients with chronic pulmonary tuberculosis were studied with pulmonary function tests before and after one-stage apical-posterior thoracoplasty with extraperiosteal paraffin plombage. The mean maximal breathing capacity (MBC) preoperatively was 83.20 L./min. (range: 37–152 L./min.), which represented a mean of 76 per cent of predicted normal (range: 27–126 per cent). The mean vital capacity (VC) preoperatively was 3.05 L. (range: 1.30–5.27 L.), which represented a mean of 80.2 per cent of predicted normal (range: 41–125 per cent). The mean loss of MBC as a result of the operation was 10.2 per cent of predicted (range: 0–35 per cent), and of VC 9.3 per cent of predicted (range: 0–35 per cent). When expressed as a percentage of preoperative MBC, the mean loss due to surgery was 13.5 per cent (range: 0–46 per cent).

Functional residual capacity (FRC), total lung capacity (TLC), and residual volume (RV) were measured preoperatively and postoperatively in 31 patients. The TLC was within normal limits in 13 patients, reduced in 7 patients, and elevated in 11 patients preoperatively. After surgery, 6 patients showed an increase in TLC of more than 200 cc. (range: 230 to 800 cc.). This increase in TLC was due entirely to an increase in residual volume. In 17 patients TLC was decreased by more than 200 cc. (range: 220 to 2,130 cc.). In 15 of these 17 patients, the decrease in TLC was caused primarily by loss of VC, although only one showed a significantly increased RV. In the remaining 14 patients the RV was either unchanged or diminished. In 8 of the 31 patients the TLC was unchanged, but in 4 of these 8 the VC was decreased and RV increased. Thus, in the entire group of 31 patients the RV was increased significantly in 8 patients, decreased in 10, and unchanged in 13.

Loss of MBC and VC and changes in RV and TLC were correlated with the size of the thoracoplasty as estimated from frontal and lateral chest roentgenograms and from the weight of the paraffin plombage. No significant correlation was found, except that the loss of MBC rose sharply when the collapse extended below the sixth posterior rib as seen in the frontal inspiratory roentgenogram. There was also no correlation between the above parameters and the estimated amount of disease in the lung tissue collapsed by the operation.

An exercise of moderate severity (O_2 uptake 400–1,300 cc. minute) was performed preoperatively and postoperatively, using a one-step test in 83 of 93 patients. The preoperative oxygen ventilation equivalent (O_2 — V) was higher than our normal for hospitalized patients (20–33 L. vent/L. O_2 uptake) with a mean of 38 L. vent/L. O_2 uptake (range: 28–50 L. vent/L. O_2 uptake). The O_2 — V increased postoperatively by more than 5 L./L. O_2 uptake in 19 patients and decreased by more than 5 L./L. O_2 in 5 patients. The increase in mean dyspnea index from 39.6 per cent preoperatively to 48.1 per cent postoperatively was thus seen to be due primarily to a diminution in MBC.

The one-second VC expressed as a percentage of determined VC was less than 73 per cent in 36 cases and more than 73 per cent in 33 cases. After an operation there was a decrease of more than 10 per cent in the one-second VC in 13 patients and an increase of more than 10 per cent in 9 patients.

Most patients showing a decrease in one-second VC after surgery had a preoperative one-second VC that was within the normal range and showed very little change in total determined VC as a result of surgery. Those showing an increase in one-second VC were primarily in the group of patients with a diminished preoperative one-second VC, and generally had a diminution of total determined VC as a result of surgery. Thus, it seems unlikely that the increase in one-second VC can be taken as a measure of lessened airway obstruction in this second group. Losses of MBC and VC are being analyzed in relation to the pres-

ence of airway obstruction preoperatively as determined by the preoperative one-second VC.

Differential bronchospirometry with measurements of ventilation, O_2 uptake, and VC was performed before surgery in 67 patients and was also carried out in 33 of these 67 postoperatively. The results of this study have been compared with similar studies on the physiologic effects of classical thoracoplasty and other types of thoracoplasty with extraperiosteal plombage.

Cavernostomy—A Salvage Procedure for Giant Tuberculous Cavities

JOHN N. BRIGGS, Encino, California, and ROBERT R. TORREY, Los Angeles, California

Chemotherapy has been responsible for the abandonment of several time-honored surgical procedures used in the treatment of cavitary pulmonary tuberculosis. Cavernostomy is among these. It is now rarely used as a salvage procedure. The cavernostomy referred to in this paper is open-cavity drainage performed in one or two stages, not the Monaldi procedure. The majority of large tuberculous cavities are now treated by pulmonary resection. There remains, however, a small percentage of patients with giant cavities that are not surgically resectable. These patients are salvageable with the judicious use of cavernostomy.

Only 14 cavernostomies have been done at the Olive View Hospital in the eleven years from 1948 to 1959. This paper reviews the indications, results, morbidity, and deaths that occurred in these 14 patients. Although this small series is not statistically significant, the results further justify the use of cavernostomy as a salvage procedure in drug-resistant patients with giant cavities who are not candidates for pulmonary resection or collapse procedures. There is an appreciable morbidity associated with cavernostomy. All 14 patients had postoperative fever for two to 30 days. In one instance, there was a postoperative pneumothorax, and one patient had hemorrhage from the cavernostomy. There were 3 deaths in the series; all were late, occurring from 10 to 22 months after operation, and none could be attributed to the operation. The deaths resulted from congestive heart failure, bronchogenic carcinoma, and progressive tuberculosis. Seven of the patients were discharged from the hospital. Six of these had noninfectious sputum, and only one had infectious sputum. Three patients continue to be hospitalized, improved, and with noninfectious sputum. One patient remains in the hospital unimproved, with sputum positive for *M. tuberculosis*.

Pulmonary Resection for Emphysematous Bullae

DAVID M. SENSENIG and THEODORE RODMAN, Philadelphia, Pennsylvania

A study, including preoperative and postoperative pulmonary function tests, was undertaken of patients with giant pulmonary bullae without generalized obstructive emphysema. Eight patients who underwent 13 operations were studied.

Wedge resection was performed six times in 4 patients. Two were much improved symptomatically and by pulmonary function studies. Two patients underwent unroofing of bullae on the left, followed by right upper lobectomy. Both showed slight improvement in function after the unroofing procedure. The patient who underwent segmental resection showed improved efficiency of oxygen uptake on the operated side, but a reduced vital capacity. Lobectomy was performed four times. Arterial oxygen saturation increased postoperatively but, when bronchospirometry and pneumoangiography were performed, the results were consistent with a reduced blood flow to the operated side. An increase in the residual volume to total lung capacity ratio has suggested overexpansion of the remaining lung tissue.

The best results were achieved in dyspneic patients who had minimal generalized airway obstruction.

The Surgical Management of Pulmonary Blebs and Bullae

HAROLD C. SPEAR, DEWITT C. DAUGHTRY, JOHN G. CHESNEY, and ASHER MARKS, Miami, Florida

Pulmonary blebs and bullae represent localized expressions of obstructive emphysema which, upon reaching large enough proportions to produce significant pulmonary parenchymal compression, warrant surgical attention.

The authors report their experiences with 14 such operative cases. The clinical and roentgenographic features are occasionally difficult to correlate. Pulmonary function studies are important in objective evaluation, but they cannot serve as criteria for operability, since some gratifying surgical results occur among those with the most compromised pulmonary function. Conservation of pulmonary parenchyma is the keynote of successful surgery. Operative techniques—including adjunctive tracheostomy and ventilatory assistance—complications, and results have been described. All patients in this series were improved postoperatively and maintained their improvement during the period of postoperative follow-up.

Coal Miners' Pneumoconio-tuberculosis—Results of Medical and Surgical Therapy

CHARLES GERNEZ-RIEUX, C. VOISIN, and P. FOURNIER, Lille, France

A total of 236 tuberculous coal miners have received combined antituberculosis drugs (streptomycin, isoniazid, and PAS) for six months to two years, followed by a double-drug regimen (PAS and isoniazid) for long-term chemotherapy. The results are poor: cavity closure and sputum conversion occurred in only 49 cases, and there were five relapses.

In tuberculous coal miners exposed to pneumoconiotic risk for ten years or more without showing a roentgenographically specific pneumoconiotic pattern, the percentage of good results is half as much as for similar male tuberculous patients without occupational exposure.

The failure of the medical treatment justifies surgical therapy; 122 pulmonary resections were realized. The results are as follows: 116 immediately favorable results and 6 deaths. Among the 78 patients followed up regularly, 62 are healed, 11 present active tuberculous lesions, 5 are dead.

These facts and the frequency of severe pulmonary lesions caused by atypical mycobacteria among coal miners point out the importance of the host in pneumoconio-tuberculosis.

Present Status of the Treatment of Bronchiectasis

WILLIAM A. HOPKINS, M. BEDFORD DAVIS, and WILLIAM C. WANSKER, Atlanta, Georgia

Bronchiectasis continues to be a disease of considerable importance, especially to the thoracic surgeon. Although surgical intervention is not indicated in all cases, it plays the major role in the treatment of this disease. Many patients who have had surgical treatment of bronchiectasis fail to have complete remission of their symptoms. This is distressing both to the patient and to the surgeon handling the case. This report deals with an analysis of 500 patients treated consecutively by the same surgical group. A careful analysis of the causes of failure has been completed.

The following causes have been found to be the major factors in the failure to relieve symptoms after surgical treatment of bronchiectasis: residual disease, postoperative atelectasis, empyema and/or bronchopleural fistula, thrombosis of residual segmental vein, associated bronchospastic disease, age of patient, stump problems (length, infection, suture abscess, etc.), and space problems.

Adequate preoperative and postoperative management of the patient may prevent many failures. Careful endoscopic and bronchographic study of the lungs is imperative for success. Failure to interpret bronchograms adequately was a common cause for leaving a diseased segment. It is believed that segmental resection has definitely increased the postoperative morbidity and, therefore, has been instrumental in the production of symptoms after surgery.

5B Immunology

An Evaluation of the Agar Double-Diffusion Test in 600 Consecutive Patients

WILLIAM LESTER, Chicago, Illinois, ROSEMARY COLTON, Hinsdale, Illinois, and ROBERT C. PARLETT and GUY P. YOUMANS, Chicago, Illinois

Serum from 600 patients admitted consecutively to the Suburban Cook County Tuberculosis Hospital-Sanitarium were studied for the presence of mycobacterial antibodies by means of the agar double-diffusion precipitation test. In this series there were 369 patients with bacteriologically confirmed diagnoses of tuberculosis, and 364 of them (98 per cent) were found to be antibody positive. Of 58 cases of photo-chromogenic infection, 53 of them (91 per cent) were antibody positive; and in 15 cases of the Battey type of infection, all were antibody positive. Antibody was demonstrable in only 25 of 31 patients (80 per cent) from whom only scotochromogenic cultures had been isolated, and the same result was obtained in 107 patients from whom no positive cultures were obtained (80 per cent had demonstrable antibodies).

Review of the data indicated that the concentration of antibody detectable by the gel-diffusion test tends in most cases to increase with the passage of time and the institution of chemotherapy. Thus, a single negative test may not be significant unless confirmed on repetition. However, the data reported indicate that a positive test demonstrating detectable antibody is of significance and does not vary appreciably on repetition.

The Effect of Homogenates Prepared from Organs of Normal and Immunized Guinea Pigs on the Respiration of Virulent Tubercle Bacilli

ANNE S. YOUMANS and GUY P. YOUMANS, Chicago, Illinois

At regular intervals following vaccination with BCG, appropriate numbers of immunized and nonimmunized guinea pigs were sacrificed, and the lungs, livers, and spleens removed and homogenized. Standard Warburg manometric techniques were used, and the effect of the organ homogenates on the respiration of virulent tubercle bacilli of the H37Rv strain was measured.

The results showed that there was a gradual increase only in the lungs of immunized guinea pigs of a substance or of substances that markedly inhibited the endogenous respiration of virulent tubercle bacilli, whereas lungs obtained from nonimmunized guinea pigs produced homogenates that stimulated the respiration of virulent tubercle bacilli. The homogenates prepared from the lungs of immunized guinea pigs also had the capacity to inhibit the stimulation of respiration of virulent tubercle bacilli produced by lactate and pyruvate.

The respiration-inhibiting factor was nondialyzable and unaffected by heating at 60°C. for 30 minutes. It could be frozen or lyophilized without loss of activity.

Immediate Component in Tuberculin Reactions

WILLIAM D. MCKEE and CUTTING B. FAVOUR, Palo Alto, California

Previous studies have shown a correlation between hyperglobulinemia and necrosis in tuberculin hypersensitivity. This study suggests that necrosis in the delayed reaction may be mediated by serum globulin.

Guinea pigs vaccinated with killed tubercle bacilli were injected intracutaneously with a 0.09 per cent suspension of autoclaved yeast particles on both flanks to produce moderate inflammation. Twenty-four hours later an intracutaneous injection of saline or histoplasmin was superimposed on the control site, and 5 γ of PPD on the test site. The control site demonstrated only mild inflammation. Within one or two hours an immediate reaction was produced in the test site with peripheral edema. The presence of necrosis in an ordinary skin test employing 5 γ of PPD was correlated with the intensity of this immediate reaction and specifically with central blanching and hemorrhage. Hypersensitive animals with no necrosis in the PPD control test produced no blanching or hemorrhage. Under identical conditions normal animals showed only mild inflammation in the test sites.

It may be postulated that a factor associated with hypersensitive serum globulin is involved in an immediate response to tuberculin.

A Study of the Heaf Test in Comparison with the Mantoux Test and the Patch Test

KATHERINE H. K. HSU and AUSTIN E. HILL, Houston, Texas

Two elementary schools in Houston were chosen for this study. Each child was given all three tests: 5 TU of PPD Mantoux test, Heaf test, and patch test. Each kind of test was performed by the same tester throughout the study. All three tests were read three times—after 48 hours, 96 hours, and seven days—by the same reader throughout the study. A total of 1,736 children completed their tests.

When the tests were read after 96 hours, the Heaf test missed only two of the 53 positive Mantoux reactors, whereas the patch test missed 24 of the 53. Reading on the seventh day did not pick up any more positive Mantoux reactors. However, among the negative 5 TU reactors there were 66 children showing mild Heaf reactions of 4–6 papules on the seventh day. Therefore, for detection of 5 TU reactors, reading the Heaf test after 96 hours is recommended.

In the second school, 250 TU of PPD were given to all children who were negative to 5 TU. Among the 1,080 children tested, only 16 showed positive reactions to 5 TU, whereas 229 were positive to 250 TU. The source for this low-grade tuberculin sensitivity will be studied by the use of antigens prepared from atypical mycobacteria.

Variations in the Endurance of Positive Mantoux Reactions in Primary Childhood Tuberculosis

WILLIAM CURTIS ADAMS, Louisville, Kentucky

Findings suggest that persistence of a positive Mantoux reaction in children with active primary tuberculosis is variable.

Seventy-nine children with active primary tuberculosis have been followed for three to five years to date as part of the U. S. Public Health Service's Tuberculosis-Meningitis-Prophylaxis Study. Repeated Mantoux tests (PPD unitage recorded) of each individual were performed by a trained team of physicians; only one observer interpreted all of the reactions. It became apparent that the positive Mantoux tests of some children reverted to negative. Thereafter, Mantoux tests were simultaneously performed in duplicate, employing corresponding doses of USPHS PPD-S and a freshly prepared commercial solution of PPD. The expected 90 per cent correlation between the duplicate Mantoux tests was observed. Unexpectedly, 58.2 per cent of the 79 children with active primary tuberculosis were observed to shift from positive to negative Mantoux reactions. These findings occurred in the control group as well as in the isoniazid prophylaxis group. Reversions occurred in patients whose initial positive reactions ranged from 5 to 20 mm. of induration.

Epidemiologic Factors in Tuberculosis—A Twenty-Four-Year Study

L. D. ZEIDBERG, R. S. GASS, R. H. HUTCHESON, and ANN DILLON, Nashville, Tennessee

This is the final report on an epidemiologic study of tuberculosis that was conducted in rural,

agricultural, Williamson County, Tennessee, for 24 consecutive years. The data comprise observations of 4,399 individuals who contributed 32,175.25 person-years of experience as members of households in which they were exposed to a known tuberculous individual, the "index case."

The prevalence of tuberculous infection and disease, attack rates, and death rates among household members have been analyzed according to age, sex, race, socio-economic status, relationship to the index case, and the sputum status of the index case. Analyses have also been made of the influence of pregnancy and the postpartum period on attack rates in child-bearing household members compared with the rates in other females of similar age.

Preliminary conclusions indicate that under similar conditions of exposure the prevalence of infection apparently is not influenced by such factors as sex, race, or relationship to the index case, but attack rates do show significant differences by these attributes. The evidence suggests that the exposure factor in the household is of prime importance in determining who is selected for attack, although a hereditary constitutional factor appears to have some influence too. The role of the socio-economic factors has not been conclusively shown at this writing, but the impression is that among people living in a rural, agricultural environment their effect may be relatively minor. The value of observing contacts to tuberculous individuals over a long period of time is demonstrated in the long lag between first exposure and development of disease in some individuals. Further importance of contact observation is shown by a comparison of the stage of disease on first diagnosis of index cases and of new cases in the households. Among the former, tuberculosis was almost invariably moderately or far advanced when it was discovered, but the new cases were predominantly in the minimal stage.

Study of an Outbreak of Tuberculosis aboard the USS Lowe

JESS W. BROMLEY, Oakland, California

Between July and September, 1959, 9 patients with pulmonary tuberculosis were admitted to the U.S. Naval Hospital, Oakland, California, from the USS Lowe (DER-325). The first patient was symptomatic and had bilateral, moderately advanced cavitary disease. All 133 members of the ship's company were studied with 70-mm. photofluorograms, and one additional case was found. This individual had bilateral, moderately advanced disease with two tension cavities. Both patients had sputum in which acid-fast bacilli were easily demonstrated on direct smear. Subsequently, the entire crew of 133 men were tuberculin tested with 5 tuberculin units of PPD-S; 75 of them had been similarly tested previously, with 4 (5 per cent) reported positive. Skin-testing and repeat skin-testing gave the following results: Of the 133 individuals tested, 99 (74 per cent) were positive, and of the 99, 65 (49 per cent) were known converters. The tuberculin-positive individuals received postero-anterior stereo and lateral chest X-rays. Six patients with early minimal infiltrates and one with a pleural effusion were discovered. Seven of the 9 patients admitted had bacteriologically proved disease, and one of the 7 developed a spontaneous pneumothorax.

A concurrent control study aboard the USS Koiner (DER-331), a ship very similar to the USS Lowe, was made. The 146 men from the Koiner were given the tuberculin test, which resulted in 27 (18 per cent) positive reactions, with one known converter. Of the total complement of 146, 84 had previously received the tuberculin test, with 4 (5 per cent of those tested) reported positive. Active pulmonary disease was not found.

The men of the two ships were compared on the basis of rate, rank, years of service, and tuberculin reaction. The sleeping compartments, ventilating systems, sanitation, and working and social associations aboard the ships were carefully evaluated. The members of the control group aboard the second ship who were tuberculin positive were almost all over the age of 30 with more than ten years of active duty, whereas most of the converters and those with active disease from the USS Lowe had had less than five years of service.

The study clearly reveals a high degree of contagion aboard a small ship with close living and working conditions and without evidence of breakdown in sanitation or in annual photofluorographic procedures. An incidence of active tuberculosis of approximately 10 per cent among tuberculin converters is demonstrated. The study shows the danger of having individuals with sputum positive for acid-fast bacilli aboard a small ship, and points out the inadequacy of photofluorograms as a case-finding technique in such a situation.

7A Surgery

Vascular Invasion in Lung Cancer: Clinicopathologic Significance

JACK M. MOSELY and DELBERT R. DICKSON, Santa Barbara, California

Surveys of carcinoma of the lung have traditionally placed the major emphasis upon the involvement of the hilar node and the cell type as the major factors in the prognosis of the disease.

It is the purpose of this presentation to show that vascular invasion is the most important single factor affecting survival in lung cancer.

Fifty cases have been extensively reviewed. Of these, 25 represent surgical resections for carcinoma of the lung, and 25 are autopsy cases of patients who died of carcinoma of the lung. A careful search has been made of all slides of numerous sections with evidence of vascular invasion in cancer of the lung. Hematoxylin-eosin stains and elastic stains have been used in the microscopic study of these cases.

An over-all incidence of venous invasion was found in 72 per cent of the surgical cases. Arterial invasion was found in 52 per cent of the resected specimens. Whereas lymphatic vessels showed invasion in 76 per cent, lymph node involvement was found to be present in 40 per cent. Of the squamous cell carcinomas, venous invasion was found in 80 per cent, and adenocarcinoma exhibited venous invasion in 75 per cent. The undifferentiated carcinomas showed 100 per cent venous invasion.

In the autopsy series, venous invasion was found in all patients who died of carcinoma of the lung.

Of the 25 surgical patients whose slides showed invasion of the pulmonary vein by carcinoma cells and who underwent resection for carcinoma of the lung, only one is alive, well, and free of disease at this time.

Isolated Perfusion of the Left Lung

J. Kenneth Jacobs, John Flexner, and H. William Scott, Jr., Nashville, Tennessee

Since the introduction of isolated perfusion techniques in 1957, methods have been described for the perfusion of various organs. The previously described technique for the lungs has necessitated the perfusion of both lungs and the use of two extracorporeal circulatory systems.

The present study was undertaken to develop a simple technique for the perfusion of one lung. After preliminary trials the following method was devised: Using a left thoracotomy, the investigator dissects the left hilar structures, opens the pericardium, and mobilizes the left atrium. He heparinizes the experimental animal, passes an occlusive tape around the left pulmonary artery, and inserts a catheter into the artery distal to the tape. He places a Satinsky clamp across the left atrium in such a manner that the flow from the left pulmonary veins can be drained via a left atrial cannula without impeding right pulmonary venous return and its flow through the mitral valve. Left pulmonary venous return is drained by gravity into a reservoir containing 500 ml. of blood, from which the left pulmonary artery is perfused with a Sigmamotor pump. Extracorporeal oxygenation is not required as the inflated left lung serves as its own oxygenator.

In these experiments perfusion was maintained for approximately 25 minutes at the rate of 250 ml. to 350 ml. per minute. To the perfusate was added nitrogen mustard in a concentration of 0.6 mg./kg. and a 5 ml. aliquot of RISA®. Radioactivity counts of the perfused and systemic blood as well as weekly complete blood counts were made to check the escape of the perfusate into the general circulation.

Diagnosis of Acute Pulmonary Embolism by Arteriography

William S. Stoney and Jesse E. Adams, Nashville, Tennessee

The Trendelenburg operation for removal of a pulmonary embolus has been almost completely abandoned because of the high mortality rate associated with the procedure and the difficulty in differentiating thromboembolism from other acute circulatory conditions. The present study was undertaken to develop a simple method of establishing the presence and location of a pulmonary embolus.

In a series of 20 dogs a large thrombus was formed in the interior vena cava and then released. A pulmonary arteriogram was obtained a short time later by injection of Hypaque® into the external jugular vein using a single-exposure portable X-ray technique. A pulmonary embolectomy was then performed.

The arteriogram obtained in this manner demonstrated the presence and precise location of the embolus in each experiment. Fifty per cent of the animals survived the embolectomy.

Bronchial Arterial Collaterals—Effect of Experimental Pulmonary Artery Ligation and Subsequent Reanastomosis

Harvey W. Kausel, L. H. S. Van Mierop, Allan S. Peck, Allan Stranahan, and Ralph D. Alley, Albany, New York

A large bronchial arterial collateral circulation can be induced experimentally in dogs and other species by ligating a major pulmonary artery. The development of bronchial arterial collaterals is paralleled by a regression in the size of the pulmonary artery and its branches distal to the ligature. The volume of collateral flow through a dog's lung several months after pulmonary arterial ligation has been studied by Liebow *et al.*, and found to approach 800 to 900 ml./M²/min. after 18

months. Despite the demonstration of precapillary communications between the expanded bronchial arterial collaterals and the pulmonary artery bed, the pressure in the pulmonary artery, distal to the ligature, remains lower than the pressure in the main pulmonary artery.

The present report describes the anatomic fate of expanded bronchial arterial collaterals induced by pulmonary arterial ligation after reanastomosis of the pulmonary artery and the functional significance of the collateral circulation after reanastomosis of the pulmonary artery. The contributions of this study, involving the use of approximately 200 dogs during the past four years, are as follows:

The difficult technical task of reanastomosing a chronically ligated pulmonary artery has been solved.

After pulmonary arterial reanastomosis, regression of the bronchial arterial collaterals occurs, provided one of two conditions is met: (*1*) reanastomosis is performed before sufficient time has elapsed to witness a marked disparity in size between the two pulmonary arteries, or (*2*) at the time of reanastomosis, the contralateral pulmonary artery is narrowed by a band to approximate the caliber of the reanastomosed pulmonary artery.

Even two years after pulmonary arterial ligation, the bronchopulmonary arterial communications in the experimental preparation are of insufficient size to set up a hemodynamic block or reverse flow when continuity of the ligated artery is restored.

Topographic Large-Section Study of Satellite Nodules in Pulmonary Coccidioidomycosis

P. J. Melnick and John D. Steele,
Los Angeles, California

Topographic large sections were made through the entire lobe in 31 resected cavitary cases of pulmonary coccidioidomycosis. Satellite nodules were found in most of the sections, often at a considerable distance from the cavity. In 12 cases the satellite nodules were large enough to be palpable, but in the majority of cases they were microscopic in size and could not be palpated. There is some difference of opinion about the satellite nodules: whether they should be removed together with the main lesion by lobectomy or whether they are successfully controlled by the host tissue reaction. Fourteen cases of nodular resection were studied with many special stains to evaluate healing and nonhealing. The satellite nodules in 11 resected cavitary cases were studied with special stains as well as with histochemical enzyme techniques.

Differing histologic and enzyme patterns were found in the healing and nonhealing nodules. In the nodules that were healing, an abundance of metachromatic acid mucopolysaccharide, reticulin fibers, collagen, esterase, alkaline phosphatase, lipase, phosphamidase, and succinic dehydrogenase were found. In the nonhealing nodules there was a marked decrease or absence of all of the above. Viable spherules in the nonhealing nodules contained endospores and various enzymes; in the healing nodules the dead spherules were transformed into empty shells without endospores or enzymes.

Coccidioidomycosis Surgical Data from the VA-AF Cooperative Study

David Salkin, San Fernando, California

Preliminary data from the four-year retrospective (1955–1958) study shows that, of 716 patients hospitalized during this period, 199 patients (26 per cent) had primary pulmonary resectional surgery. Two hundred resections were done (one bilateral) on 113 cavitary, 84 nodular, and 3 pneumonic lesions. The operations included 64 subsegmental, 75 segmental, and 61 lobar resections.

There were 25 significant complications, of which 9 (4.5 per cent) were due to *C. immitis* and 16 (8 per cent) due to other causes. The important factors in the coccidioidomycotic complications were cavitary lesions, extensive resections, remaining palpable disease, and whether the surgery was done by study or nonstudy units.

Comparison with tuberculosis surgery shows that the complication rate of resection is similar. It appears that modern surgery, based upon careful selection of cases and operative management, is much safer than in previous years.

7B Nontuberculous Lung Disease

Rheumatoid Pleurisy with Effusion

David T. Carr and John G. Mayne,
Rochester, Minnesota

Pleurisy with effusion is an uncommon manifestation of rheumatoid arthritis. We have recently observed a series of 9 such cases and have prepared a clinical study of these patients with particular emphasis on the characteristics of the pleural disease. The examinations of the pleural fluid pointed up certain aids in diagnosis; for instance, a low concentration of glucose in the pleural fluid is usually present in these patients. The pleura was biopsied in 2 cases; the histologic picture of the pleural disease has been described. Follow-up data have been prepared.

An Evaluation of Pleural Fluid Glucose Determinations

A. H. Russakoff, Birmingham, Alabama, and Charles A. LeMaistre and Hal J. Dewlett, Dallas, Texas

It is timely that there be an appraisal of pleural fluid sugar determinations as an adjunct in the diagnosis of "idiopathic pleural effusion." The literature is contradictory as to its value. A careful review of the data reported by numerous authors discloses a lack of uniformity in methodology, thus making impossible both an evaluation of each individual study as well as a composite analysis.

Taking a heterogeneous group of patients with undiagnosed pleural effusions—later found to be tuberculous, nontuberculous, and undiagnosed—the authors ran simultaneous glucose tolerance tests on the blood and on the pleural fluid. No complications ensued. The classical Folin-Wu method was used.

The results of this study indicate the following:

The previously reported literature on the subject is virtually without value in determining the place of pleural fluid glucose determinations as an adjunct in the diagnosis of pleural effusion.

There is a propitious and specific time for doing a thorancentesis in order to carry out pleural fluid glucose determinations. The tap should be done in the morning after an overnight fast.

A fasting pleural fluid sugar of less than 60 mg. per 100 ml. is suggestive evidence of a tuberculous process. Levels in excess of 60 mg. per 100 ml. are much more suggestive of a nontuberculous etiology.

In many instances the level of the pleural fluid glucose exceeds the level of glucose in the blood at certain points, indicating that glucose is sequestered into the pleural fluid. Thus there is no correlation between the levels of glucose in the blood and in the pleural fluid.

The practice of doing simultaneous determinations of the glucose in the blood and in the pleural fluid at any time other than in the fasting state is ungrounded, without meaning, and misguiding.

Microscopic Examination of Bronchial Epithelium in Children

Oscar Auerbach, Arthur Purdy Stout, E. Cuyler Hammond, and Lawrence Garfinkel, East Orange, New Jersey

Fifty microscopic slides were prepared from the tracheobronchial tree removed at autopsy from each of 35 children under the age of 15 for a total of 1,750 slides. As a control, 1,656 slides were prepared from the smaller branches of the bronchial trees of 36 adults. The total 3,406 slides were mixed in completely random order and identified only by a code number. Each slide was examined microscopically and a record made of all changes found in the epithelium.

In the great majority of slides from children, the epithelium was found to be completely normal. In those not completely normal, the most frequent finding was regeneration. No carcinoma *in situ* was found in slides from children.

Observations on the Evolution and Course of Sarcoidosis

David Reisner, Hines, Illinois

A group of 86 consecutive, histologically confirmed cases of sarcoidosis form the basis for this study. Of these, 69 patients had extended follow-up, and two thirds were observed for periods ranging from 5 to 29 years.

The course of the disease was extremely variable, ranging from apparent full recovery to fatal termination. Favorable outcome was three times as frequent in patients with disease of recent origin as in those with disease of chronic duration. Differences in the character of the disease and source of selection of the clinical material are, in large measure, responsible for some of the seemingly contradictory views regarding the course and prognosis of sarcoidosis.

On the basis of observations on the consecutive manifestations and course of the disease, it is possible to distinguish the following broad phases in the evolution of sarcoidosis: an early or "primary" phase, characterized by conspicuous lymphadenopathy, with particular predilection for thoracic lymph nodes; a phase of dissemination, of varying extent and degree; a phase of progressive and apparently irreversible organic changes, resulting in functional damage and chronic disability. Spontaneous regression is a prominent feature of the first two phases.

Complicating tuberculosis is a major factor responsible for the unfavorable outcome in the present series.

A Reactant to Human Organ Extracts in Patients with Certain Pulmonary Diseases and Rheumatoid Arthritis

James F. Hammarsten, Allen R. Hennes, Margaret Z. Moore, and R. L. Carpenter, Oklahoma City, Oklahoma

Serum from 61 of 90 patients with active tuberculosis and 15 of 30 patients with idiopathic ob-

structive emphysema agglutinated latex particles coated with human lung extract. Agglutination took place in six of seven specimens of serum from patients with rheumatoid arthritis. In contrast, serum from only one of 45 control subjects agglutinated these particles.

An apparent inhibitor of this reaction was removed by heating serum for 15 minutes at 65° C.

Reactant in serum from patients with tuberculosis and emphysema is absorbed best by lung and kidney, relatively little by liver, and not at all by serum, although some differences exist in the absorption pattern between these two diseases. In contrast, reactant in rheumatoid serum is absorbed best by liver and serum, relatively little by kidney, and not at all by lung.

A trypsin digest supernatant of lung removes reactant from emphysema serum but not from rheumatoid serum.

The reactant in serum from patients with emphysema and tuberculosis is markedly different from that in rheumatoid serum and seems to be directed against a completely different organ component.

The Association of Chronic Peptic Ulcer and Chronic Pulmonary Emphysema

GEORGE L. BAUM, MARTIN H. KALSER, AND JOHN H. KATHE, Coral Gables, Florida

An increased incidence of chronic peptic ulcer in patients with chronic obstructive pulmonary emphysema has been reported by several investigators. At the authors' institution the hospital records of 478 subjects, diagnosed as having chronic pulmonary emphysema, were studied retrospectively. Chronic peptic ulcer was present in 22.7 per cent, in contrast to an incidence of 2.7 per cent of chronic peptic ulcer among 3,225 patients of an identical age group (over 40) admitted during the same study period. When exacting criteria for the diagnosis of these two diseases were applied (fluoroscopic, post-mortem, pulmonary function, surgical, or roentgenographic criteria), there were 61 unequivocal cases of chronic peptic ulcer among 208 patients with unequivocal chronic obstructive pulmonary emphysema, for an incidence of 29.3 per cent.

Studies of fasting gastric acidity in a group of 30 unselected patients with chronic obstructive pulmonary emphysema indicated a mean gastric juice pH of 2.2 mean control (10 subjects) = 4.5, and a mean gastric juice free HCl of 35.4 units mean control (10 subjects) = 15.6 units. There were no correlations between the gastric secretion pH or free HCl or free HCl and arterial blood gas or pH measurements, nor was there any significant change in the gastric acidity during a 30-minute 5 per cent CO_2 inhalation period and for 30 minutes thereafter.

Preliminary studies in a group of 5 patients with chronic pulmonary emphysema and chronic peptic ulcer reveal a mean plasma hydrocortisone level of 21.9 μ/100 ml., contrasted with values of 15.9 μ/100 ml. (5 subjects with pulmonary emphysema alone), 11.6 μ/100 ml. (6 subjects with peptic ulcer alone), and 10.9 μ/100 ml. (6 control subjects). Further work is now in progress.

It is concluded that these studies do not support the hypothesis that CO_2 retention or other blood gas abnormalities may predispose toward the development of peptic ulcer in patients with chronic pulmonary emphysema. A possible stress mechanism is suggested.

8B Immunology

An Immunogenic Tubercle Bacillus Extract

ALFRED J. CROWLE, Denver, Colorado

Repeated experiments in the writer's laboratory have shown that killed tubercle bacilli injected into mice as water-oil emulsions provoke in these animals a clear-cut resistance to challenge tuberculosis, and that this resistance can be matched by substitute treatment with some proteinaceous tubercle bacillary extracts. These bacillary constituents have been studied biochemically and immunochemically over the past three years as part of a general investigation on tuberculoimmunity induction and its mechanisms.

Of these extracts, one of the most interesting and easiest to work with has been obtained from trypsin-digested tubercle bacilli. This substance is readily soluble in buffer, giving a clear amber-colored solution that can be passed through bacterial filters efficiently and easily. It immunizes mice as effectively as similar quantities of living, avirulent tubercle bacilli or killed, virulent ones of the same strain as is used for infection. The extract does not need any potentiation for immunogenic effect in mice by the crude lipids that seem necessary for its protectiveness in guinea pigs. In fact, its immunogenicity is not disturbed by exhaustive extraction of its lipids by neutral organic solvents. It can evoke immunity as a water-oil emulsion prepared with any one of three different emulsifying agents (mannide monooleate, glycerine monooleate, Aquaphore).

A similar, but more complex, proteinaceous extract has the same immunogenic properties. Interestingly, it can be used with equal effectiveness in water-oil emulsions, simply mixed with *n*-hexadecane (the emulsion oil phase), as an alum precipitate, or merely in buffer solution.

These two substances have been submitted to preliminary chemical and immunochemical analyses. The active essence appears to be a low molecular weight polypeptide that may be susceptible to definitive purification by immunoelectrophoresis.

Experimental Killed Antituberculosis Vaccines Prepared by Solvent Extractions

ANSON HOYT, MILLIE A. THOMPSON, FREDERICK J. MOORE, AND C. RICHARD SMITH, Los Angeles, California

Tubercle bacilli contain stable immunogenic components. R. J. Anderson's purified tubercle bacillus wax after thirty years' storage has significantly immunized mice against experimental tuberculosis. Recently prepared fractions of this wax have done likewise.

Fresh BCG-IV (Rosenthal) and heated (100° C. for three hours) human *M. tuberculosis* (Lederle) were completely extracted by slightly modified methods of Anderson. Small fresh masses of H37Rv have been partially extracted and fractionation of a large H37Rv mass is under way. Many extracts and their residues have been tested for their mouse-immunizing potencies.

Results indicate that the large, neutral ethanol-ether extracts and also most firmly bound lipids are nonimmunogenic. Neutral chloroform H37Rv extracts have immunized as well, or almost as well, as live BCG. Comparable BCG extracts seem less effective. BCG residues, however, appear more immunogenic than like residues of H37Rv. Most vaccines produced from the heated bacilli have immunized poorly in tests already completed.

The Immunogenicity and Allergenicity of Mycobacterial Cell Walls and Intracellular Particles in CF_1 and Strong A Mice

KOOMI KANAI, GUY P. YOUMANS, AND ANNE S. YOUMANS, Chicago, Illinois

Small enzymatically active intracellular particles collected by ultracentrifugation from extracts of mechanically ruptured attenuated mycobacterial cells were highly immunogenic for mice. However, the question of whether these intracellular particles represent all of the immunogenic substance of the tubercle bacillus has not been resolved.

Therefore, mycobacterial cell walls have been isolated and purified and their immunogenicity compared with that of the intracellular particles, using two strains of mice that differ markedly in their immune response to whole cells.

Repeated experiments have revealed that the intracellular particles obtained from the H37Ra strain are far more immunogenic for mice than are the purified cell walls of the same mycobacterial strain. Furthermore, the immunogenicity of the intracellular particles obtained from H37Ra cells disrupted by pressure was much greater than the immunogenicity of particles obtained from cells disrupted by grinding in a ball mill or Waring Blendor with powdered glass.

Tuberculin hypersensitivity was induced in guinea pigs by the purified mycobacterial cell walls but not by the immunogenic intracellular particles.

The Influence of Alteration of the Diet on the Susceptibility of the Albino Rat to Mycobacterium Tuberculosis

RICHARD L. COSTELLO, LOYD W. HEDGECOCK, AND TOM R. HAMILTON, Kansas City, Kansas

The rat is naturally resistant to progressive, fatal tuberculosis. Only hypophysectomy, alloxan-diabetes, and injection of cortisone have been reported to lower this resistance. An increased susceptibility to *M. tuberculosis* in thiouracil-treated rats fed atherogenic dietary factors is reported herein.

Rats prefed with Purina chow, a basal unaltered synthetic ration, or the synthetic ration altered by the addition of cholesterol (5 per cent) or sodium cholate (1 per cent), remained grossly healthy for at least 60 days following infection with *M. tuberculosis* H37Rv (0.3 mg. intravenously). Animals lost their resistance when fed the synthetic diet containing either thiouracil (0.3 per cent) or thiouracil plus sodium cholate and cholesterol. The susceptibility of the latter group was especially marked. Uninfected rats fed the experimental diets remained healthy throughout the experiment.

Histopathologic pictures of sections from animals in all groups showed granulomatous changes, with cellular proliferative foci in the infected rats in all groups that were not fed thiouracil. The thiouracil-treated animals presented more widespread diffuse interstitial involvement, including an apparent increase in the number of large cells with foamy cytoplasm.

Serologic and Skin Test Activity of Polysaccharides Derived from Systemic Mycotic Pathogens

RALPH A. KNIGHT, GILBERT A. HILL, AND STANELY MARCUS, Salt Lake City, Utah

Polysaccharide antigens have been prepared from *Histoplasma capsulatum, Blastomyces derma-*

titidis, *Coccidioides immitis*, and *Sporotrichum schenckii*. The complement-fixing activity of these polysaccharides and the cell-free extract antigens from each of these fungi were compared for homologous and heterologous reactions. These findings indicated that cross reactions do occur, but that a degree of specificity could be achieved by adjusting the concentrations of the antigen and antibody. Although the cell-free extract antigens reacted at higher dilutions of the antibody, the polysaccharide antigens were active in the complement-fixing test, and the same specific reaction could be achieved with the optimal concentrations of the polysaccharide antigen and the corresponding antibody.

The skin-test activities of these polysaccharide antigens were investigated in guinea pigs and humans. A comparison of the skin-test activity of the polysaccharide antigens with standard skin-test preparations or commercially available skin-test antigens showed that the polysaccharide preparations possessed skin-test activity equal to the filtrate antigens. Cross reaction did occur at higher polysaccharide concentrations, but adjusting the concentration of the antigen produced a specific reaction. Limited data suggested that the polysaccharide antigen produced a more clear-cut area of induration and the filtrate antigen produced more erythema and less induration. Theoretically, the polysaccharide antigen possesses all the advantages advanced for the use of PPD rather than Old Tuberculin.

NOTICES

The following dates and locations of the next four annual meetings of the National Tuberculosis Association and the American Trudeau Society have been selected and approved:

1961—Cincinnati, Ohio—May 21–26
1962—Miami Beach, Florida—May 20–25
1963—Denver, Colorado—May 12–17
1964—New York, New York—May 24–29

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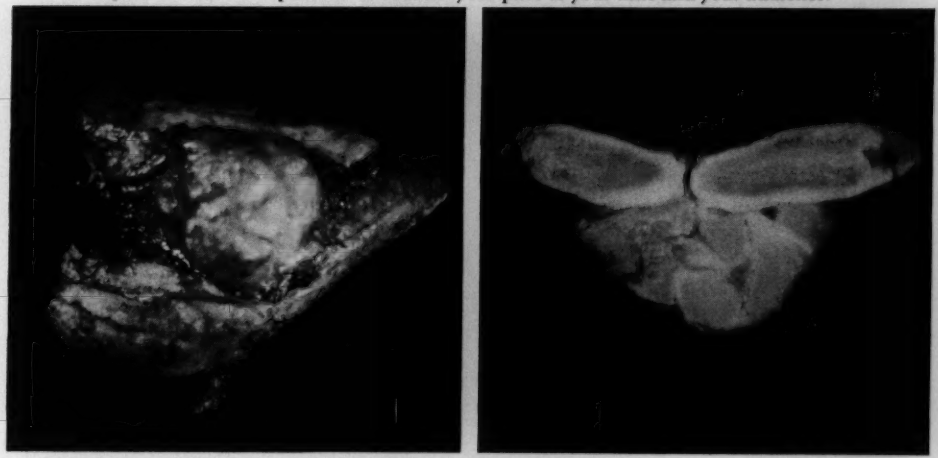

Chondroma of chest wall—2 aspects

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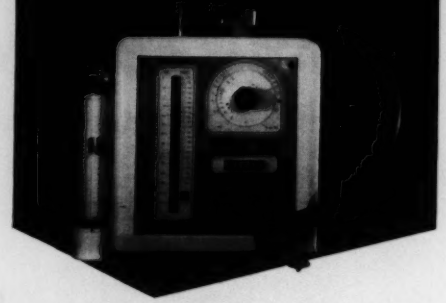

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Pulmonary specialist	24	5.50
Starred internist	85	19.49
Starred surgeon	31	7.10
Surgeon	3	.68
Starred thoracic surgeon	27	6.19
Starred radiologist	7	1.60
Starred public-health specialist	5	1.14
Starred pathologist	3	.68
Starred pediatrician	3	.68
Starred otologist	2	.45
Starred and unstarred specialists in allergy, bacteriology, gastroenterology, hospital administration, industrial medicine, OALR, OBG, orthopedic surgery, physical medicine—one each	9	2.06
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Special research	1	.22
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No age given	1	.22
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35–39	55	12.61
40–44	58	13.30
45–49	50	11.46
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100–200,000	32	7.33
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1	1	1	0	1	2	2	50–100,000
2	0	1	1	0	0	2	100–200,000
3	6	0	0	0	1	4	200–500,000
4	3	2	0	0	1	0	500–1,000,000
2	4	1	1	2	0	6	Over 1,000,000
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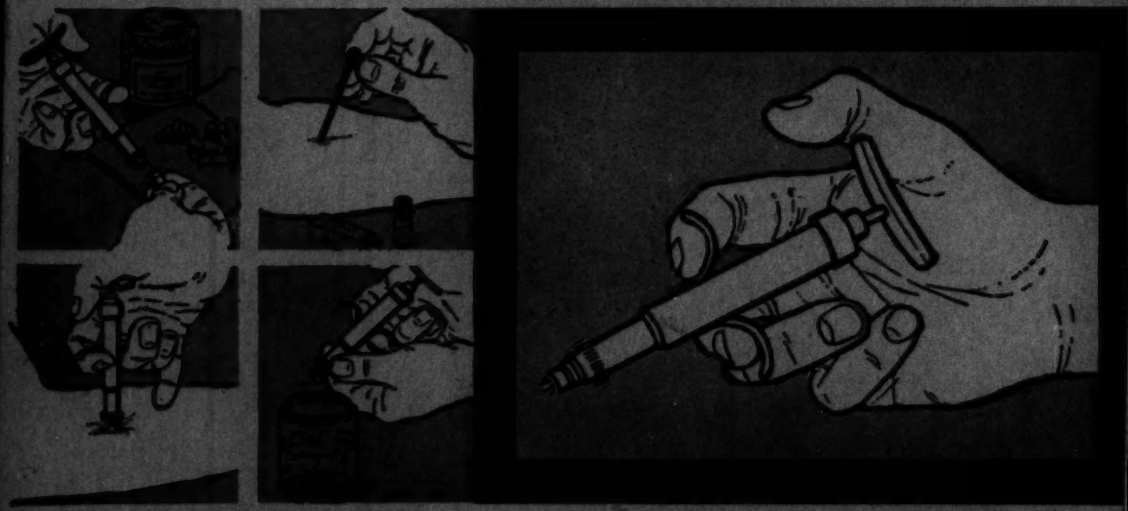

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